01-07-02 JC05 Rec'd PCT/PTO 03 JAN 2002 $ Box Seq

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 3128.1013

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR: 10/030559

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/KR00/00713	3 July 2000	5 July 1999

TITLE OF INVENTION

NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZARSUGAR AND ANTISENSE OLIGOMERS THEREOF

APPLICANT(S) FOR DO/EO/US

JUNG, Kyeong-Eun; YANG, Mirim; LEE, Kwangjun; KIM, Kichul; and LIM, Hong

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a ☒ is attached hereto (required only if not communicated by the International Bureau).
   b ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired
   d ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4))
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409)
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment
17. ☒ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1 821 - 1 825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

    Acknowledgement postcard
    Hard copy of Sequence listing (2 pages)

JC13 Rec'd PCT/PTC 03 JAN 2002

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR 10/030559	INTERNATIONAL APPLICATION NO PCT/KR00/00713	ATTORNEY'S DOCKET NUMBER 428.1013

24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS	PTO USE ONLY
☒ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1 445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**		
☐ International preliminary examination fee (37 CFR 1 482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$1,040.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	20 - 20 =	0	x $18.00	**$0.00**	
Independent claims	3 - 3 =	0	x $84.00	**$0.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,040.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2				**$0.00**	
SUBTOTAL =				**$1,040.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,040.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☒	**$40.00**	
TOTAL FEES ENCLOSED =				**$1,080.00**	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of ________ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **50-0217** in the amount of **$1,080.00** to cover the above fees A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No **50-0217** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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022856
PATENT TRADEMARK OFFICE

SIGNATURE

Michael N. Mercanti
NAME

33,966
REGISTRATION NUMBER

January 3, 2002
DATE

428.1013

UNITED STATES PATENT & TRADEMARK OFFICE

Examiner: Unknown Art Unit: Unknown

Re: Application of: JUNG, Kyeong-Eun, et. al.

Serial No.: To be assigned

Filed: herewith

For: **NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZARSUGAR AND ANTISENSE OLIGOMERS THEREOF**

PRELIMINARY AMENDMENT

Assistant Commissioner
for Patents
Washington, D.C. 20231

January 3, 2002

Sir:

Prior to the examination, please amend the above-identified patent application as follows:

IN THE SPECIFICATION:

Page 1, before line 1, please insert the following paragraph:

--This patent application claims a benefit of priority from Korean Patent Application No. 1999/26947 filed July 5, 1999, through PCT Application Serial No. PCT/KR00/00713 filed on July 3, 2000, the contents of each of which are incorporated herein by reference.--.

IN THE SEQUENCE LISTING:

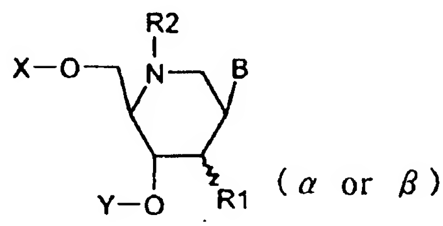
Please replace the sequence listing in the substitute specification with the new sequence listing attached hereto.

IN THE CLAIMS:

Please amend claim 1 as follows:

1. (Amended) A modified nucleotide monomer comprising: a five-membered ribose, sugar of natural nucleotide, substituted with azasugar of six-membered ring and of the formula,

Formula 1



wherein

B is a natural nucleobase or a modified nucleobase with or without a protecting group,

R1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy; α- or β-methoxyethoxy; α- or β-halogen; α- or β-aminoalkoxy; α- or β-dimethylamino-oxyalkoxy; or α- or β-O-acyl,

R2 is hydrogen; aralkyl; nitrobenzyl; haloaralkyl; cyanobenzyl; alkoxybenzyl; lower alkyl; aryl with or without a substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

X is hydrogen or hydroxy protecting group, and

Y is hydrogen, phosphate, activated phosphate, activated phosphite or solid support.

Please amend claim 2 as follows:

2. (Amended) The nucleotide monomer according to claim 1, wherein R1 is selected from the group consisting of hydrogen, methoxy, ethoxy and methoxyethoxy.

Please amend claim 3 as follows:

3. (Amended) The nucleotide monomer according to claim 1, wherein R2 is selected from the group consisting of diphenylmethyl, methyl, *t*-butyl, benzyl, cyanobenzyl, fluorobenzyl, methoxybenzyl and fluorenyl (Fmoc).

Please amend claim 4 as follows:

4. (Amended) The nucleotide monomer according to claim 1, selected from the group consisting of:

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl} adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxyethoxypiperidine-3-yl}adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethylpiperidine-3-yl}adenine;

6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine;

6-N-benzoyl-{(3S,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine;

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine;

4-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} cytosine;

2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} guanine;

{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrithyloxymethyl-4-methoxypiperidine-3-yl}thymine;

{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine;

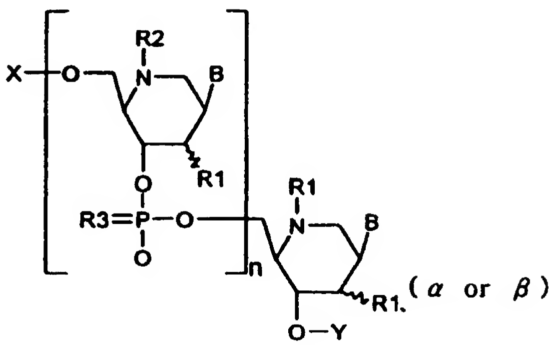
{(3R,4R,5R,6R)-N-fluorenyl-5-[(2-cyanoethoxy)N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine; and

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-isobutyryloxypiperidine-3-yl}adenine.

Please amend claim 5 as follows:

5. (Amended) Antisense oligomers represented by the formula 2, which are prepared with the nucleotide monomers of claim 1 as a part or whole of oligonucleotide,

Formula 2



wherein,

n is 0 to 30,

B is a natural nucleobase or a modified nucleobase with or without a protecting group,

R1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy; α- or β-methoxyethoxy; α- or β-halogen; α- or β-aminoalkoxy; α- or β-dimethylamino-oxyalkoxy; or α- or β-O-acyl,

R2 is hydrogen; aralkyl; haloaralkyl; cyanobenzyl; alkoxybenzyl; lower alkyl; aryl with or without a substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

R3 is oxygen or sulfur,

X is hydrogen or hydroxy protecting group, conjugate group or oligonucleotide, and

Y is hydrogen, phosphate, active phosphate, active phosphite, solid support, conjugate group or oligonucleotide.

Please amend claim 8 as follows:

8. (Amended) Chimeric oligomers, which have the monomers of claim 1 or the antisense oligomers of claim 5 at both ends of the molecules, and phosphodiesters or phosphorothioate oligonucleotides in their middle.

Claim 10 has been amended as follows:

10. (Amended) A process for preparing antisense oligomers of claim 5, which comprises:

(1) Substituting dimethoxytrityl group for a primary hydroxyl group linked to the sugar of a nucleotide monomer, phosphoramidite group for a secondary alcohol group, and protecting the nucleobases except thymine with an appropriate protecting group;

(2) Performing a condensation reaction of the monomer of step (1) linked to solid support with an oligonucleotide;

(3) Removing the solid support and protecting group from the oligomer of step (2); and

(4) Removing a 5'-hydroxy protecting group from the oligomer.

Please amend claim 12 as follows:

12. (Amended) The process for preparing antisense oligomers according to claim 10, wherein the condensation of step (2) is characterized by having a nucleotide monomer linked to the solid support at positions except 3'-terminus, prepared via standard phosphoramidite process using a DNA synthesizer.

Please amend claim 13 as follows:

13. (Amended) A pharmaceutical composition containing the antisense oligomers of claim 5 as active ingredients, which are effective for inhibition or prevention of protein syntheses.

Please amend claim 14 as follows:

14. (Amended) A pharmaceutical composition containing the antisense oligomers of claim 5 as active ingredients, which are effective for the treatment of hepatitis of viral or

bacterial origin, cancers and immune diseases.

Please add new claims 15-20:

15. (New) A pharmaceutical composition containing the chimeric oligomers of claim 8 as active ingredients, which are effective for inhibition or prevention of protein syntheses.

16. (New) A pharmaceutical composition containing the chimeric oligomers of claim 8 as active ingredients, which are effective for the treatment of hepatitis of viral or bacterial origin, cancers and immune diseases.

17. (New) A compound of claim 1, wherein R1 is α- or β-fluoro; α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino oxyethyloxy.

18. (New) A compound of claim 1, wherein R2 is methylbenzyl, ethylbenzyl, dimethylbenzyl, halodiphenylmethyl, ethoxybenzyl, ethyl, or propyl.

19. (New) A compound of claim 5, wherein R1 is α- or β-fluoro; α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino oxyethyloxy.

20. (New) A compound of claim 5, wherein R2 is methylbenzyl, ethylbenzyl, dimethylbenzyl, halodiphenylmethyl, ethoxybenzyl, ethyl, or propyl.

REMARKS

This preliminary amendment is being submitted to correct typographical errors, insert a claim of priority based on a foreign application into the specification and to conform the claim language to U.S. standards. Also note that the Title has been changed in the substitute specification to correct the misspelling of "AZARSUGAR" to "AZASUGAR." It is respectfully submitted that no new matter has been entered and that the present application is in all respects complete and in condition for favorable consideration.

Attached hereto is a marked-up version of the changes made to the claims by the preliminary amendment. The attached appendix is captioned "**<u>Version with markings to show changes made</u>**."

If the Examiner has any questions regarding the amendment presented herein, it is requested that the Examiner contact the undersigned at the telephone number shown below.

An early and favorable action on the merits is earnestly solicited.

Respectfully submitted,
ROBERTS & MERCANTI, L.L.P.

Sapna Gadhia
Reg. No. 48,978

ROBERTS & MERCANTI, L.L.P.
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Phone: 973-621-0660
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"Express Mail" mailing label no. EL 696 654 825 US
Date of Deposit <u>January 3, 2002</u>
I hereby certify that this correspondence and/or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above, in an envelope addressed to "Assistant Commissioner for Patents, Washington, DC 20231".

ROBERTS & MERCANTI, L.L.P
By.
Sapna Gadhia

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428.1013

UNITED STATES PATENT & TRADEMARK OFFICE

	Examiner:	Unknown	Art Unit:	Unknown
Re:	Application of:	JUNG, Kyeong-Eun, et. al.		
	Serial No.:	To be assigned		
	Filed:	herewith		
	For:	**NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZARSUGAR AND ANTISENSE OLIGOMERS THEREOF**		

APPENDIX I
VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Page 1, before line 1, please insert the following paragraph:

--This patent application claims a benefit of priority from Korean Patent Application No. 1999/26947 filed July 5, 1999, through PCT Application Serial No. PCT/KR00/00713 filed July 3, 2000, the contents of each of which are incorporated herein by reference.--.

IN THE SEQUENCE LISTING:

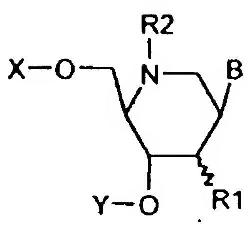
Please replace the sequence listing in the substitute specification with the new sequence listing attached hereto.

IN THE CLAIMS:

Claim 1 has been amended as follows:

1. (Amended) <u>A</u> [Modified] <u>modified</u> nucleotide [monomers] <u>monomer comprising:</u> [represented by the formula 1, in which] <u>a</u> five-membered ribose, sugar of natural nucleotide, [is] substituted with azasugar of six-membered ring <u>and of the formula,</u>

Formula 1



(α or β)

[Wherein] wherein

[(1)] B is a natural nucleobase or a modified nucleobase with or without a protecting group,

[(2) R^1] R1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy [such as α- or β-methoxy, or α- or β-ethoxy]; α- or β-methoxyethoxy; α- or β-halogen [such as α- or β-fluoro]; α- or β-aminoalkoxy [such as α- or β-aminomethoxy or α- or β-aminoethoxy]; α- or β-dimethylamino-oxyalkoxy [such as α- or β-dimethylamino oxyethyloxy]; or α- or β-O-acyl,

[(3)] R^2] R2 is hydrogen; [araalkyl] aralkyl [such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl]; nitrobenzyl; [haloaraalkyl] haloaralkyl [such as fluorobenzyl]; cyanobenzyl; [alcoxybenzyl] alkoxybenzyl [such as methoxybenzyl or ethoxybenzyl]; lower [molecular] alkyl [such as methyl, ethyl, propyl or tertbutyl]; aryl with or without a substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

[(4)] X is hydrogen or hydroxy protecting group, and

[(5)] Y is hydrogen, phosphate, activated phosphate, activated phosphite or solid support.

Claim 2 has been amended as follows:

2. (Amended) The nucleotide [monomers] monomer according to claim 1, wherein R1 is selected from the group consisting of hydrogen, methoxy, ethoxy and methoxyethoxy.

Claim 3 has been amended as follows:

3. (Amended) The nucleotide [monomers] monomer according to claim 1, wherein R2 is selected from the group consisting of diphenylmethyl, methyl, *t*-butyl, benzyl, cyanobenzyl,

fluorobenzyl, methoxybenzyl and fluorenyl (Fmoc).

Claim 4 has been amended as follows:

4. (Amended) The nucleotide [monomers] <u>monomer</u> according to claim 1, [which are represented by the formula 1 and is] selected from the group [comprising] <u>consisting of</u>:

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine [(the compound of Example 1)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl} adenine [(the compound of Example 2)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxyethoxypiperidine-3-yl}adenine [(the compound of Example 3)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethylpiperidine-3-yl}adenine [(the compound of Example 4)];

6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine [(the compound of Example 5)];

6-N-benzoyl-{(3S,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine [(the compound of Example 6)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine [(the compound of Example 7)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine [(the compound of Example 8)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

[(the compound of Example 9)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine [(the compound of Example 10)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine [(the compound of Example 11)];

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine [(the compound of Example 12)];

4-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} cytosine [(the compound of Example 13)];

2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} guanine [(the compound of Example 14)];

{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrithyloxymethyl-4-methoxypiperidine-3-yl}thymine [(the compound of Example 15)];

{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine [(the compound of Example 16)];

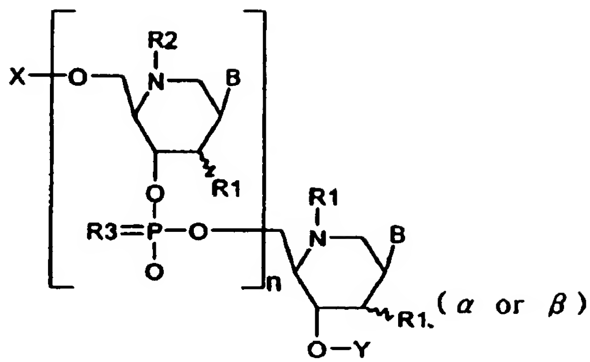
{(3R,4R,5R,6R)-N-fluorenyl-5-[(2-cyanoethoxy)N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine [(the compound of Example 17)]; and

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-isobutyryloxypiperidine-3-yl}adenine [(the compound of Example 18);] .

Claim 5 has been amended as follows:

5. (Amended) Antisense oligomers represented by the formula 2, which are prepared with the nucleotide monomers of claim 1 as a part or whole of oligonucleotide[.] ,

Formula 2



[Wherein] wherein,

n is 0 to 30,

[(1)] B is a natural nucleobase or a modified nucleobase with or without a protecting group,

[(2) R^1] R1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy [such as α- or β-methoxy, or α- or β-ethoxy]; α- or β-methoxyethoxy; α- or β-halogen [such as α- or β-fluoro]; α- or β-aminoalkoxy [such as α- or β-aminomethoxy or α- or β-aminoethoxy]; α- or β-dimethylamino-oxyalkoxy [such as α- or β-dimethylamino oxyethyloxy]; or α- or β-O-acyl,

[(3) R^2] R2 is hydrogen; [araalkyl] aralkyl [such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl]; [haloaraalkyl] haloaralkyl [such as fluorobenzyl]; cyanobenzyl; [alcoxybenzyl] alkoxybenzyl [such as methoxybenzyl or ethoxybenzyl]; lower [molecular] alkyl [such as methyl, ethyl, propyl or tertbutyl]; aryl with or without a substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

[(4) R^3] R3 is oxygen or sulfur,

[(5)] X is hydrogen or hydroxy protecting group, conjugate group or oligonucleotide, and

[(6)] Y is hydrogen, [phophate] phosphate, active phosphate, active phosphite, solid support, conjugate group or oligonucleotide.

Claim 8 has been amended as follows:

8. (Amended) Chimeric oligomers, which have the monomers of claim 1 or the antisense oligomers of claim 5 at both ends of the molecules, and phosphodiesters or [phosphothioate] phosphorothioate oligonucleotides in their middle.

Claim 10 has been amended as follows:

10. (Amended) A process for preparing antisense oligomers of claim 5, which comprises:

(1) Substituting [dimethoxytrithyl] dimethoxytrityl group for a primary hydroxyl group linked to the sugar of a nucleotide monomer, phosphoramidite group for a secondary alcohol group, and protecting the nucleobases except thymine[,] with an appropriate protecting group;

(2) Performing a condensation reaction of the monomer of step (1) linked to solid support with [a] an oligonucleotide;

(3) Removing the solid support and protecting group from the oligomer of step (2); and

(4) Removing a 5'-hydroxy protecting group from the oligomer.

Claim 12 has been amended as follows:

12. (Amended) The process for preparing antisense oligomers according to claim 10, wherein the condensation of step (2) is characterized by having a nucleotide monomer linked to the solid support at positions except 3'-terminus, prepared via standard [phoshoamidite] phosphoramidite process using a DNA synthesizer.

Claim 13 has been amended as follows:

13. (Amended) A [Pharmaceutical] pharmaceutical [compositions] composition containing the antisense oligomers of claim 5 [or the chimeric oligomers of claim 8] as active ingredients, which are effective for inhibition or prevention of [proteins] protein syntheses.

Claim 14 has been amended as follows:

14. (Amended) A [Pharmaceutical] pharmaceutical [compositions] composition containing the antisense oligomers of claim 5 [or the chimeric oligomers of claim 8] as active ingredients, which are effective for the treatment of hepatitis of viral or bacterial origin, cancers and immune diseases.

Please add new claims 15-20:

15. (New) A pharmaceutical composition containing the chimeric oligomers of claim 8 as active ingredients, which are effective for inhibition or prevention of protein syntheses.

16. (New) A pharmaceutical composition containing the chimeric oligomers of claim 8 as active ingredients, which are effective for the treatment of hepatitis of viral or bacterial origin, cancers and immune diseases.

17. (New) A compound of claim 1, wherein R1 is α- or β-fluoro; α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino oxyethyloxy.

18. (New) A compound of claim 1, wherein R2 is methylbenzyl, ethylbenzyl, dimethylbenzyl, halodiphenylmethyl, ethoxybenzyl, ethyl, or propyl.

19. (New) A compound of claim 5, wherein R1 is α- or β-fluoro; α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino oxyethyloxy.

20. (New) A compound of claim 5, wherein R2 is methylbenzyl, ethylbenzyl, dimethylbenzyl, halodiphenylmethyl, ethoxybenzyl, ethyl, or propyl.

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By S. Gadhia
Sapna Gadhia

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UNITED STATES PATENT & TRADEMARK OFFICE

	Examiner:	Unknown	Art Unit:	Unknown
Re:	Application of:	JUNG, Kyeong-Eun, et. al.		
	Serial No.:	To be assigned		
	Filed:	herewith		
	For:	**NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZARSUGAR AND ANTISENSE OLIGOMERS THEREOF THEREOF**		

STATEMENT OF ACCURACY OF SUBSTITUTE SPECIFICATION
(37 C.F.R. §§1.125)

Assistant Commissioner
for Patents
Washington, D.C. 20231

January 3, 2002

Sir:

I, the below named Attorney of Record, hereby state:

the attached Substitute Specification contains no new matter. A marked up version showing all the changes in red ink to the specification of record is also enclosed.

Respectfully submitted,

ROBERTS & MERCANTI, L.L.P.

By:
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Invention: NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZARSUGAR AND ANTISENSE OLIGOMERS THEREOF

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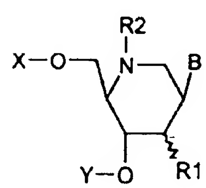
NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZASUGAR AND ANTISENSE OLIGOMERS THEREOF

FIELD OF THE INVENTION

The present invention relates to a nucleotide monomer represented by the formula 1 in which a five-membered ribose is substituted with a six-membered azasugar, antisense oligomers represented by the formula 2, and process for preparation thereof.

The antisense oligomers of the present invention are useful for developing antisense drugs since they have high binding affinity to mRNA, good membrane permeability and improved resistance to nuclease.

<FORMULA 1>



(α or β)

wherein,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower

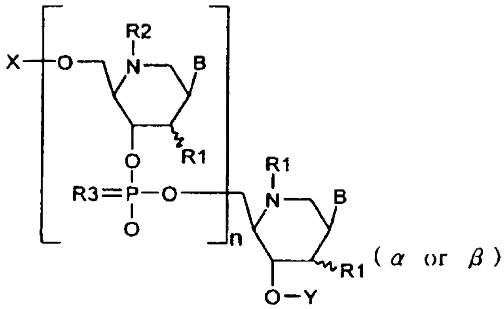
molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; aralkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaralkyl such as fluorobenzyl; cyanobenzyl; alkoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

(4) X is hydrogen or hydroxyl protecting group, and

(5) Y is hydrogen, phosphate, activated phosphate, activated phosphite or solid support.

<FORMULA 2>



wherein,

n is 0 to 30,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; aralkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaralkyl such as fluorobenzyl; cyanobenzyl; alkoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or

fluorenyl (Fmoc),

(4) R^3 is oxygen or sulfur,

(5) X is hydrogen or hydroxyl protecting group, conjugate group or oligonucleotide, and

(6) Y is hydrogen, phosphate, activated phosphate, activated phosphite, solid support, conjugate group or oligonucleotide.

BACKGROUND

Proteins comprise more than 20 amino acids and have a very complex and diverse tertiary structure, thus it is very difficult to develop a drug which selectively acts on them. There has been much progress in the development of protein inhibitors as the tertiary structures of various proteins are elucidated by computer simulation and X-ray analysis. However, there has not yet been successful development of effective protein inhibitors.

On the other hand, it has been possible to develop various drugs targeting nucleic acids since nucleic acid comprises 4 different nucleotides of adenosine, guanosine, cytidine and thymidine or uridine, and has a property of complementary binding to each other (Uhlmann *et al.*, "Antisense Oligonucleotides: A New Therapeutic Principles" *Chem. Rev.,* **1990**, 90, 543-584 ; Cohen et al., "The New Genetic Medicine"

[redacted]

Scientific American, **1994**, 271, 76-82).

In vivo protein synthesis accomplishes through expression of the gene which encodes the amino acid sequence. Particularly, one strand of DNA with double-helix structure is transcribed into mRNA and the mRNA is translated to form a protein. On this basis, the drugs aiming at the nucleic acids have been developed and they contain an oligonucleotide with complementary sequence to the mRNA.

The oligonucleotide can bind to the complementary nucleotide of mRNA, inhibit its translation to the protein, and block or reduce the formation of disease-causing proteins. Because the oligonucleotide sequence is reverse (antisense) to the genetic information sequence (sense), the drug is named as antisense drug and the technique antisense technique.

In the late 1970's, Stephenson and Zamecnik found that a synthetic DNA fragment can inhibit the synthesis of viral proteins (Stephenson et al., *Proc. Natl. Acad. Sci. USA,* **1977**, 95, 285 ; Zamecnik et al., *Natl. Acad. Sci. USA,* **1977**, 95, 280). In 1980's, it was also reported that the antisense RNA is synthesized in vivo can regulates the gene expression (Simons et al., *Cell,* **1983**, 34, 683 ; Mizuno et al., *Natl. Acad. Sci. USA,* **1984**, 81, 1966).

However, these natural type oligonucleotides are easily degraded by a nuclease in the body, and a sufficient pharmacological effect can not be expected in spite of their antisense effect. There has been active research effect to produce antisense drugs with improved stability by modifying the structure of antisense oligomers.

The 1st generation antisense drugs are oligomers with phosphate linkage replaced by other groups such as phosphorothioate, methylphosphate, etc. The phosphorothioate is an oligomer whose oxygen of the phosphate group is replaced by sulfur, and it has lower binding affinity to mRNA than the natural type of DNA. However, the phosphorothioate oligomers show strong pharmacological activity *in vivo* or *in vitro*. Some of the 1st generation antisense drugs are being clinically tested as anti-viral or anti-cancer agents and some others are commercially available as anti-viral agents (Bennett et al., "Antisense oligonucleotides: is the glass half full or half empty" *Biochem. Pharmacol.* **1998**, 55, 9-19).

However, these phosphorothioate oligomers also have side effects of toxicity and undesirable immune response (Stein et al., *Current Opinion in Oncology*, **1994**, 6, 587-594 ; Krieg et al, *Nature*, **1995**, 374, 549 ; O'Brien et al., *Leukemia*, **1994**, 8, 2156). New

strategy has been to develop the antisense drugs without these problems and is based on replacing phosphate backbone of the oligonucleotide by amide or ether, modifying the structure of base or ribose (De Mesmaeker et al., "Antisense Oligonucleotides" *Acc. Chem. Res.* **1995**, 28, 366-374).

The 2nd generation antisense drugs are oligomers with modified sugar in the oligonucleotides. They include oligomers containing ribose with methoxy, methoxyethoxy (Martin et al., *Helv. Chim. Acta,* **1995**, 186, 584) or aminoalkoxy (Griffey et al., *J. Med. Chem.* **1996**, 39, 5100-5109) group at 2′ position, oligomers containing hexose (Herdewijn et al., In Carbohydrate Modifications in Antisense Research; ACS Symposium Series 580; Sanghvi, Y. S., Cook, P. D., Eds.; American Chemical Society: Washington, DC, 1994; pp 80-99), oligomers containing pentose (Moser et al., Strategies and Chemical Approaches toward Oligonucleotide Therapeutics. In Perspectives in Medicinal Chemistry; Testa, B. et al., Eds.; *Verlag Helvetica Chimica Acta*: Basel, 1993, pp 275-97), oligomers containing 4′-aminoribose (Scharer et al., *J. Am. Chem. Soc.* **1995**, 117, 6623-6624), oligomers containing a 4′-thiobase (Bellon *et al.*, 4-Thio RNA: a novel class of sugar-modified B-RNA. In Carbohydrate Modifications in Antisense Research; ACS Symposium Series 580; Sanghvi, Y. S., Cook, P. D., Eds.; American Chemical Society:

Washington, DC, 1994; pp 68-79) and their derivatives.

mRNA and DNA having the complementary sequence to each other exist in duplex (double strand) form at an ambient temperature (or body temperature). However, they are separated into single strands as the temperature increases, the extent of which is measured following the change in UV absorbance. As the temperature increases, the UV absorbance increases representing sigmoidal curve due to the increase in the amount of single strands whose UV absorbance is higher than that of the duplex. Tm (melting temperature) is defined as the temperature at which 2nd derivative of the sigmoidal type curve is zero.

High Tm value of the oligomer to mRNA represents high binding affinity to RNA, and is regarded as a very important factor for the antisense molecules. The binding affinity to mRNA has been measured for the oligomers with various substituents at 2′ position by several researchers (Breslauer et al., *Proc. Natl. Acad. Sci. USA,* **1986**, 83, 3740 ; Freier et al., *Nucleic Acids Res.* **1997**, 25, 4429-4443).

Among the 2nd generation antisense drugs, the oligomers comprising a replaced base with methoxy or fluoro group at 2′ position have high Tm values because electronegative groups introduced at 2′ position increase the binding affinity of the oligomers to RNA

(Kawasaki et al., *J. Med. Chem.,* **1993**, 36, 831-841).

In addition, oligomers with alkoxy group such as methoxy at 2′ position have improved resistance to nuclease when compared with the natural type of DNA. The chimeric oligomer consisted of the modified nucleotides with phosphorothioate backbone and the 2′-methoxy substituent shows lower toxicity than that of the 1st generation antisense oligomers (Lesnik et al., *Biochemistry,* **1993**, 32, 7832 ; Milligan et al., *J. Med. Chem.* **1993**, 36, 1923), and its clinical testing was started in 1997 (Agrawal et al., *Curr. Opin. Chem. Biol.*, **1998**, 2, 519).

On the other hand, the oligonucleotide has a potential as diagnostic reagent for genetic deficiencies, primer for PCR, etc. (Englisch et al., *Ang. Chem. Int. Ed.* **1991**, 30, 613-629). It may also be useful for the investigation of the secondary structure of protein and the relationship between protein structure and its activity (Verma et al., "Modified Oligonucleotides", *Ann. Rev. Biochem.*, **1998**, 67, 99-14).

These inventors of the present invention have developed a novel antisense drug with high binding affinity to mRNA, and improved resistance to nuclease resulting in good stability. The oligomer of the present invention contains six-membered azasugar as a

basic sugar unit substituting the five-membered ribose and shows high binding affinity and good stability.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a nucleotide monomer with six-membered azasugar replacing five-membered ribose which shows high binding affinity to mRNA and improved resistance to nuclease.

It is a further object of this invention to provide antisense oligomer comprising the nucleotide monomer with azasugar unit partially or as a whole.

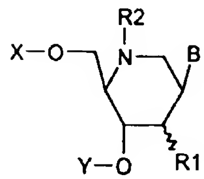
It is an additional object of this invention to provide process for preparing the nucleotide monomer represented by the formula 1 and the antisense oligomer represented by the formula 2.

Further features of the present invention will appear hereinafter.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides a nucleotide monomer with six-membered azasugar replacing a five-membered ribose represented by the formula 1 and process of preparation thereof.

<FORMULA 1>


(α or β)

wherein,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; aralkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaralkyl such as fluorobenzyl; cyanobenzyl; alkoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl (Fmoc),

(4) X is hydrogen or hydroxyl protecting group,

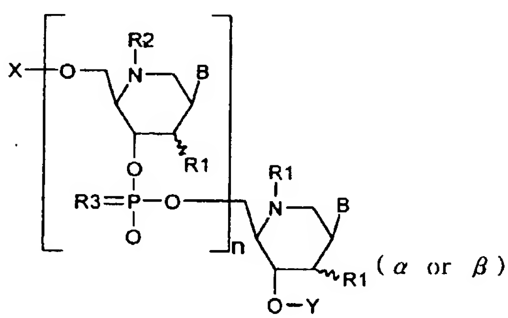
and

(5) Y is hydrogen, phosphate, activated phosphate, activated phosphite or solid support.

Particularly, R^1 is preferably β-methoxy, or β-ethoxy and R^2 is preferably diphenylmethyl.

In addition, the present invention provides an antisense oligomer represented by the formula 2 which contains the above-mentioned nucleotide monomer partially or as a whole, and process of preparation thereof.

<FORMULA 2>



wherein,

n is 0 to 30,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-

ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; aralkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaralkyl such as fluorobenzyl; cyanobenzyl; alkoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl (Fmoc),

(4) R^3 is oxygen or sulfur,

(5) X is hydrogen or hydroxyl protecting group, conjugate group or oligonucleotide, and

(6) Y is hydrogen, phosphate, activated phosphate, activated phosphite, solid support, conjugate group or oligonucleotide.

Particularly, it is preferably R^1 is β-methoxy, β-ethoxy and R^2 is diphenylmethyl.

In the formula 2, n is 1 to 30 including both upper and lower nucleotides, and is preferably 6 to 21. Properties of the oligomer do not depend on the distribution of abovementioned nucleotide monomer in the molecule. However, for the increased binding

affinity, it is desirable to have nucleotides at least 3 bases apart rather than in sequence.

Also, the present invention provides pharmaceutical compositions for effective inhibition of the protein synthesis, which comprises the nucleotide monomer, the antisense oligomer or the chimeric oligomer as an active ingredient.

The present invention also provides the pharmaceutical compositions containing the nucleotide monomer, the antisense oligomer or chimeric oligomer as an active ingredient which is effective for the treatment of hepatitis, cancer or immune diseases by infection of virus or bacteria.

Hereinafter, the present invention is described in detail.

In the present invention, the lower molecular alkyl is defined as an alkyl group containing 1-4 carbon atoms and includes methyl, ethyl, propyl, isopropyl, butyl, etc.

The lower molecular alkoxy is an alkoxy group containing 1-4 carbon atoms, includes epoxy, propoxy, butoxy, isopropoxy, etc, and is preferably methoxy or ethoxy.

O-acyl is O-acetyl, O-ethylcarbonyl, O-

propylcarbonyl, etc, aryl is an aromatic hydrocarbon with or without substituents including phenyl, paranitrophenyl and parabromophenyl, aralkyl is alkyl having an aryl group which contains benzyl, ethylphenyl and diphenylmethyl, and is preferably diphenylmethyl.

Heteroaryl is the five-membered or the six-membered ring having one or more of a heteroatom such as sulfur or nitrogen, examples of which are 4-pyridyl and 3-thiophen. Heteroalkyl is alkyl having the five-membered or the six-membered ring having one more of a heteroatom such as sulfur and nitrogen, an example of which includes 4-pyridylmethyl.

The hydroxyl protecting group is one generally known to protect hydroxyl group which includes a 4,4'-dimethoxytrityl group, a lower molecular alkanol, a trimethylsilyl ether (TMS ether), tetra-butyldimethyl silyl ether (TBDMS ether), and is preferably a 4,4'-dimethyltrityl group.

The nucleobase is any natural or modified nucleobases and is preferably a natural nucleobase such as adenine, cytosine, guanine, thymine and uracil or a modified nucleobase with the protecting group, which includes N-benzoiladenine, N-benzoilcytosine and N-isobutyrylguanine. Of the modified nucleobase, 5-(1-propynyl) uracil, 5(1-propynyl)cytosine, inosine, 5-methylicytosine and 2,6-diaminopurine are used commonly.

The oligonucleotide is the natural oligonucleotide

of 1-30 sugar units or its phosphorothioate derivatives.

The solid support may be selected from a controlled pore glass (CPG, in Oligonucleotide synthesis, a practical approach, *M. J. Gait ed.*, Oxford:IRS press, 1984), an oxalyl controlled pore glass(Alul et al., *Nucleic Acids Res.* 1991, 19, 1527), a TentaGel support (Wright et al., *Tetrahedron Lett.* 1993, 34, 3373) composed of aminopolyethyleneglycol derivatives, and a Poros which is a copolymer of polystyrene/divinylbenzene. It is preferably a CPG.

The conjugate group is a group which is bound to the primary or the secondary hydroxyl group via a covalent bond, and promotes absorption of the oligomer. It is includes cholesterol, polylysine, phospholipids, biotin, polyethylene glycol, phenanthroline, phenazine, phenanthridin, anthraquinone, acridine, fluorescein, rhodamine, coumarine and dyes.

The present invention provides a process of preparation for the nucleotide monomer with the six-membered azasugar. The nucleotide monomer of the present invention can be prepared by a process represented by the reaction schemes 1-5.

The reaction scheme 1 is a process for preparation of the oligomer which comprises the steps of preparing the basic six-membered azasugar and

binding the adenine nucleobase to a sugar.

The reaction scheme 2 is a process for condensation of the other nucleobase, thymine, cytosine and guanine.

The reaction scheme 3 is a process for binding the various groups to nitrogen of the azasugar.

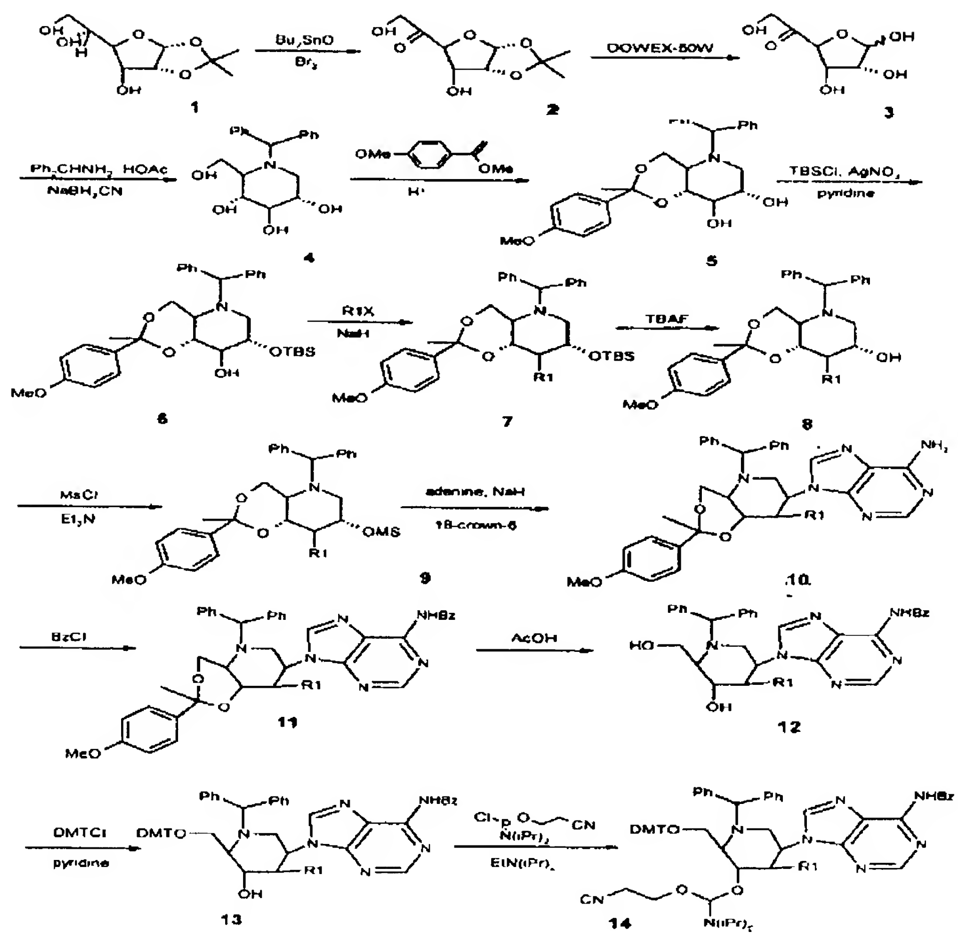
The reaction scheme 4 is a process for removing the hydroxyl group at C-4 of the azasugar via reduction.

The reaction scheme 5 is a process for changing the synthetic pathway to produce hydroxyl group with opposite stereochemistry.

According to the synthetic pathways represented by reaction scheme 1-5, it is possible to synthesis the monomer represented by the formula 1 which contains all compounds in Examples.

The following explains the reaction scheme 1-5 in more detail.

<REACTION SCHEME 1>



wherein, R^1 is the same as defined in the formula 1.

The reaction scheme 1 is the process for the preparation of the six-membered azasugar. A deoxynojirimycin derivative (the compound 4 of the reaction scheme 1) with azasugar as a basic backbone is

synthesized by the known methods using commercially available glucose derivative (Baxter et al., *J. Org. Chem.*, **1994**, 59, 3175-3185).

All intermediates and final products synthesized in the following steps are newly made compounds.

A ketone compound 2 is prepared by oxidation of glucose, compound 1 with a protecting group using dibutyltinoxide (Bu_2SnO) and bromine (Br_2), and the protecting group of the compound 2 is removed with acidic resins to obtain compound 3.

Diphenylmethylamine was added to protect for the compound 3, and ring-shaped six-membered azasugar 4 was obtained. Compound 5 is obtained by protecting both the primary hydroxyl group at 6′ position and the secondary hydroxyl group at 5′ position of sugar with α,ρ-dimethoxystyrene.

For the protection of a diol, various other agents beside α,ρ-dimethoxystyrene may be used. However, with other reagents, the reaction does not proceed or the recovery of the product is reduced.

Since the styrene protecting group has a chiral center, the compound 5 is obtained as mixture of 2 diastereomers. However, there is no need to separate the diastereomers because they become one compound in the following deprotection step of styrene protecting group to get compound 12.

Compound 6 protected with

tertbutyldimethyldisilyl (TBS) selectively at the less crowded secondary hydroxyl group of C-3 is synthesized by the reaction of the compound 5 with the tert-butyldimethyl-silyl chloride (TBSCl), $AgNO_3$ and pyridine.

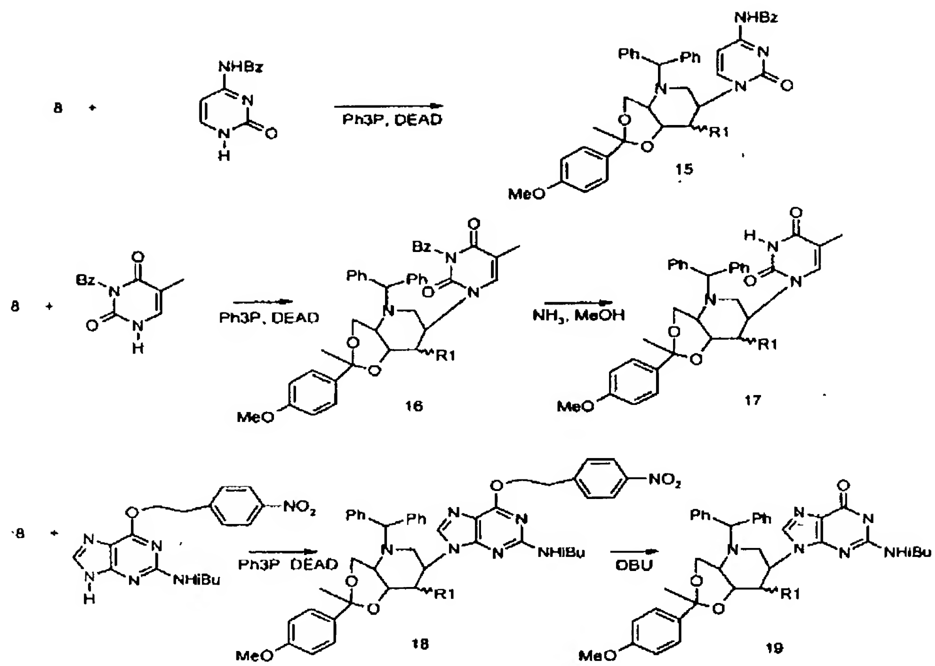
Compound 7 is obtained by alkylating the compound 6 with alkylhalide and NaH, and compound 8 is prepared by removing the TBS protecting group at 3′ position with the reaction of tertbutylammonium fluoride(TBAF).

Compound 9 is obtained by methane sulfonylation of the hydroxyl group of compound 8, and compound 10, a nucleoside, is prepared by condensation with adenine using sodium hydride and 18-crown-6.

Compound 11, a nucleoside, is prepared by monobenzoylation of the amine group of adenine, and compound 12 is prepared by removing the protecting group at 5′ and 6′ hydroxyl groups with 80% acetic acid.

Compound 13 is obtained by treating the nucleoside 12 with 4,4′-dimethoxytrityl chloride, and a phosphoramidite compound 14 is prepared by treating with 2-cyanoetyldiisopropylchlorophosphoramidite ($ClP(OCH_2CH_2CN)N(iPr)_2$) and diisopropylethylamine ($EtN(iPr)_2$).

<REACTION SCHEME 2>



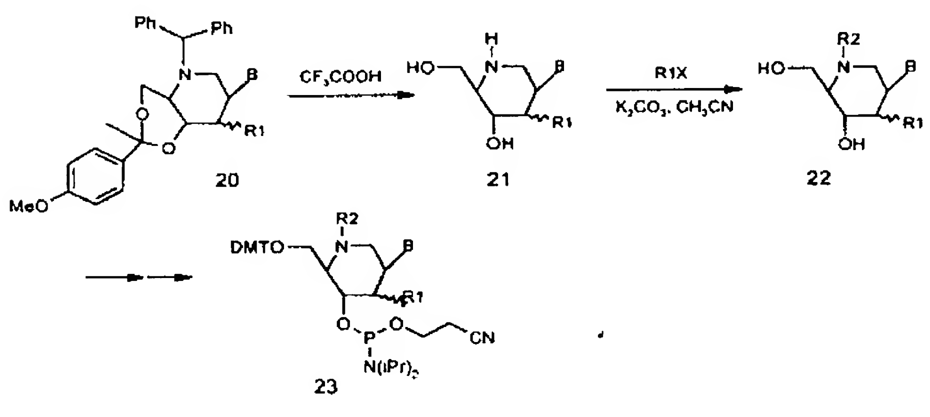
wherein, R^1 is the same as defined the formula 1.

The reaction scheme 2 represents the synthetic pathways for the condensation of azasugar with other nucleobases (cytosine, thymine, and guanine) except adenine. The process in the scheme 1 may be used, but their reaction yields are low and there is possibility of forming isomers. It is desirable to use Mitsunobu method according to the reaction scheme 2 for the condensation of cytosine, thymine and guanine monomer.

Compound 8 of reaction scheme 1 is allowed to react with N-benzoylcytosine, N-benzoylthymine, and N^2-

isobutyryl-O^6[2-(p-nitrophenyl)ethyl]guanine to form the corresponding condensation products in the presence of triphenylphosphine and DEAD (diethyl azodicarboxylate) in the tetrahydrofuran as solvent. Benzoylthymidine derivative 16 is converted into thymidine derivative 17 by removing the benzoly protecting group with ammonia gas. Compound 19 is synthesized by removing the carbonyl protecting group of guanosine derivative 18 using DBU (1,8-diazabicycle[5.4.0]undec-7-ene). Compounds 15, 17 and 19 are used to form monomers which may be utilized for the synthesis of oligomers either as in the reaction scheme 1 or as in the reaction scheme 3 shown in the following.

<REACTION SCHEME 3>

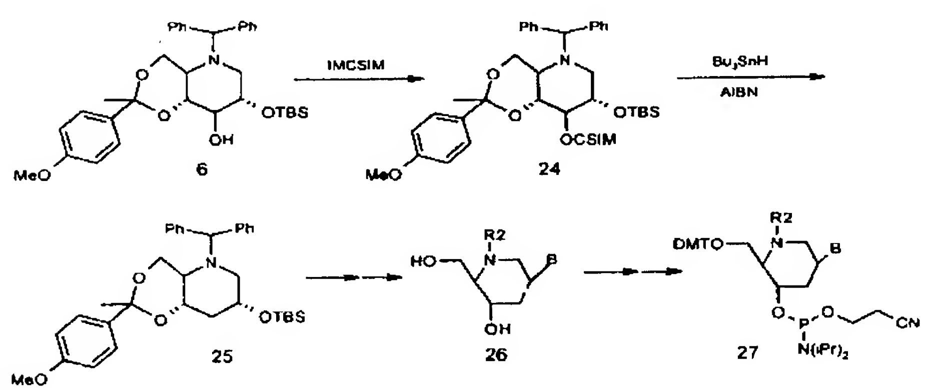


wherein, R^1, R^2 and B is the same as defined the formula 1

The reaction scheme 3 represents the process for introducing various R^2 groups at the nitrogen of six-membered sugar ring of the nucleoside derivatives, which are obtained in the reaction scheme 1 or 2.

Compound 21 is formed when compound 20 is treated with trifluoroacetic acid (CF_3COOH). Compound 22 is synthesized by alkylation of the azasugar with alkylhalide in the presence of potassium carbonate, or triethylamine or dimethylamino pyridine (DMAP). Compound 23 is synthesized by the same steps as in the reaction scheme 1.

<REACTION SCHEME 4>

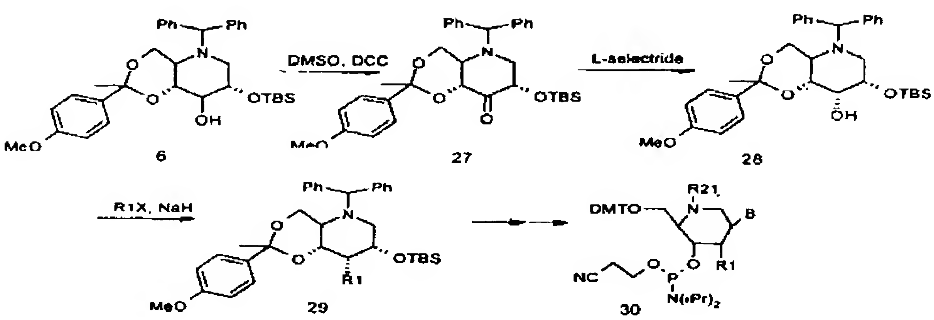


wherein, R^2 and B is the same as defined the

formula 1

The reaction scheme 4 is a process for removing the hydroxyl group at C-4 of azasugar by reduction. Compound 24 is prepared by the reaction of azasugar compound 6 with thiocarbondiimidazole, and compound 25 is synthesized by reduction with tributyltin hydride (nBu_3SnH). A compound 26 and 27 are synthesized as in the steps of the reaction scheme 1, 2 or 3.

<REACTION SCHEME 5>



wherein, R^1, R^2 and B is the same as defined the formula 1

The reaction scheme 5 is the process for altering the stereochemistry of the hydroxyl group at position 4 of azasugar. Compound 27 is obtained from compound 6 according to the oxidation reaction of Swern. Compound

28, having the hydroxyl group with opposite orientation to that of compound 6, is obtained by the reduction reaction of compound 27 with L-selectride. Compound 29 is obtained by alkylation of compound 28, and a phosphoramidite compound 30 is obtained by the reaction cited in the previous schemes.

The present invention provides the process for preparing antisense oligomers, a part or whole of which is composed of nucleotide monomers with six-membered azasugar instead of ribose, a natural five-membered sugar.

The oligomer of the present invention is prepared by solid phase or liquid phase method, solid phase method being preferable. The details of the oligonucleotide synthesis with the solid phase method are described in "Oligonucleotide Synthesis, A Practical Approach", Gait (ed.), IRL Press, Washington D. C. (1984), Caruthers and et al., U.S. Pat. No.4,458,066 and 4,500,707.

In order to prepare the oligomer via condensation reaction of the nucleotide monomer of the present invention, there is need to protect one primary hydroxyl group of the nucleotide sugar with a dimethoxytrityl group, one secondary hydroxyl group with phosphoramidite group, and nucleobases except thymine with other suitable protecting groups.

To introduce the monomer of the formula 1 at 3′-position of the oligonucleotide, it needs to be secured at a solid support. The monomer is transformed to a hemisuccinate using a controlled pore glass (CPG) with amino group, which can be purchased. Then, the reaction is completed by condensation with mesitylene-2-sulfonyl chloride/1-methyl-1H-imidazole.

To introduce the monomer of the formula 1 at the other positions except the 3′-end of the oligonucleotide, the standard phosphoramidite process is performed using DNA synthesizer (For example, ABI 392). Generally, concentration of the monomer with formula 1 and its reaction time with solid resins are the same as those of common phosphoramidite process of DNA synthesizer. However, if the groups other than hydrogen are present at 2′ position, the condensation reaction time of the monomer is needed to be extended to 600 from 60 sec in the original reaction.

Once the desired oligomer is produced, the solid support and the protecting group need to be removed, which may be done simultaneously or in two separation steps. In general, the solid support and the protecting group are removed by the treatment with ammonium hydroxide at ambient temperature (2 hours) and at 55°C (17 hours), respectively.

The protecting group for 5′-hydroxyl group of the oligomer is a dimethoxytrimethyl group, which can be

eliminated in the final step of DNA synthesis by using the program built-in the DNA synthesizer or by treating with 80% acetic acid, dichloroacetic acid or trichloroacetic acid.

If has hydroxyl group at 2'-position, the oligomer is prepared in the DNA synthesizer using isobutyl group for the monomer preparation, which is removed by ammonia in the late stage of the general DNA synthesis process. For the nitrogen of azasugar of the monomer, fluorenyl group (F-moc) is used for protection and the elimination is carried out by usual method.

In the process of the present invention to prepare the monomer and the antisense oligomer, the present inventors make the process simple by attaching the nucleobases to 3'-carbon position of the six-membered azasugar (piperidine). If the base is introduced at the aminal position, the reaction product would be α- and β-mixture, requiring separation. Using the process of the invention, the present inventors are able to produce the desired nucleotide with the base only at the carbon position, not at the aminal position. In addition, for the introduction of various group at the nitrogen position, the present inventors make use of azasugar which can be easily modified without the trouble of using strong basic reagents. The oligomers with various group introduced at nitrogen position

usually have a high Tm to RNA, indicating high affinity to mRNA, and may prove to be effective antisense drugs. The membrane permeability is improved when the present inventors use six-membered azasugar, substituting ribose, to which hydrophobic group is attached.

The oligomer composed of carbocyclic nucleotides is known to be resistant to nuclease activities. Also, the antisense oligomer of the present invention with phosphate groups is replaced by phosphorothioate partially or as a whole, and has both increased binding affinity and the increased stability to nuclease.

The nucleotide and the antisense oligomer of the present invention can be also prepared in the form of chimeric oligomer which contains phosphodiester or phosphorothioate oligonucleotide in the middle of the molecule.

As the cited above, the present invention provides the nucleotide monomer having the six-membered azasugar replaced by the natural five-membered ribose and the novel oligomer composed of the nucleotide monomer partially or as a whole. The modified nucleotide can bind more strongly to the target RNA than the natural type of DNA, and it also has higher resistance to the degrading enzyme nuclease. It has improved cell membrane permeability when the lipophilic group is introduced at the nitrogen position of

azasugar.

The oligomer of the present invention can be used for any application requiring antisense oligonucleotide, and it has good characteristics as antisense drugs because of its high binding affinity to mRNA.

When the disease-inducing proteins and the genes involved in their synthesis are discovered, the antisense treatment drugs can be developed by designing to act selectively on the important part (4-30 bases) such as an initiator codon (Akhtar et al, *Nature Genetics*, **1993**, 4, 215). The site of drug action on the mRNA can be determined by several methods (Mishra et al, *C. R. Acad. Sci. III*, 1994, 317, 977 ; Milner et al, *Nat. Biotechnol.*, **1997**, 15, 537). However, it often shows a good antisense effect when the initiation codon or the transcriptional start site is selected as the attacking site (Bacon et al, *Oncogene Res.*, **1991**, 6, 13).

If the sequence of the target mRNA for the antisense oligomer is not known, it can be determined by the analysis of the protein sequence using the genetic code. If the protein sequence is not known, the protein can be used purified and its sequence determined by the known methods.

In addition, the mRNA or DNA responsible for the protein synthesis may be isolated and identified using

the known methods, and the sequences thereof can be used for developing the antisense drugs.

The antisense oligomer compatible with the mRNA sequence is preferably composed of 4-30 units of the monomer, and more preferably 7-22 units.

The oligomer of the present invention can bind to DNA or RNA of various cells including normal cells, cancer cells, tumor cells, protoplasmic cells, amorphous cells and virus. The binding sequences are bacterial sequences, viral sequences, cancer cell sequences and chromosomal sequences. The binding of the oligomer of the present invention to DNA or RNA can inhibit protein synthesis, or can promote the specific protein synthesis by inhibiting the expression of the inhibitor protein.

In addition, the oligomer of the present invention can be used for cure of an infectious disease induced by virus or bacteria, cancer, immune diseases and coronary restenosis. The viral diseases include AIDS, hepatitis B and C, Herpes virus and cytomegalovirus. Cancer contains oncogenes such as c-myc and c-erbB-2 involved the target sequence to DNA or RNA, tumor suppress genes, protein genes-involving genes (protein kinase A, protein kinase C, c-rat kinase, bcl-2, bcr, abl, etc), and the autoimmune diseases contain rheumatoid arthritis, psoriasis, crohn disease, polyneuritis, the 1st type of diabetes mellitus and

lupus.

Moreover, the present invention provides pharmaceutical compositions containing the nucleotide monomer of the formula 1 and the antisense oligomer of the formula 2 as an active ingredient.

The nucleotide monomer, the antisense oligomer or the chimeric oligomer of the present invention can be administered orally or parenterally, and be used in general form of pharmaceutical formulation.

The compounds of the present can be prepared for oral or parenterally administration by mixing with generally-used fillers, extenders, binders, wetting agents, disintegrating agents, diluents such as surfactant, or excipients.

The present invention also includes pharmaceutical formulations in dosage units. This means that the formulations are present in the form of individual parts, for example tablets, coated tablets, capsules, pills, suppositories and ampules, the active compound content of which corresponds to a fraction or a multiple of an individual dose. The dosage units can contain, for example, 1, 2, 3 or 4 individual doses or 1/2, 1/3 or 1/4 of an individual dose. An individual dose preferably contains the amount of active compound which is administered in one application and which usually corresponds to a whole, one half, one third or

a quarter of a daily dose.

Preferred pharmaceutical formulations which may be mentioned are tablets, coated tablets, capsules, pills, granules, suppositories, solutions, suspensions and emulsions, pastes, ointments, gels, creams, lotions, dusting powders and sprays.

Solid formulations for oral administration are tablets, pill, dusting powders and capsules, liquid formulation for oral administrations are suspensions, solutions, emulsions and syrups, and the abovementioned formulations can contain various excipients such as wetting agents, sweeteners, aromatics and preservatives in addition to generally-used simple diluents such as water and liquid paraffin.

Tablets, coated tablets, capsules, pills and granules can contain the active compound or compounds in addition to the customary excipients, such as (a)fillers and extenders, for example starches, lactose, sucrose, glucose, mannitol and silicic acid, (b)binders, for example carboxymethylcellulose, alginates, gelatine and polyvinylpyrrolidone, (c)humectants, for example glycerol, (d)disintegrating agents, for example agar-agar, calcium carbonate and sodium carbonate, (e)solution retarders, for example paraffin, and (f)absorption accelerators, for example quaternary ammonium compounds, (g)wetting agents, for example cetyl alcohol and glycerol monostearate, (h)adsorbents,

for example kaolin and bentonite, and (i)lubricants, for example talc, calcium stearate, magnesium stearate, and solid polyethylene glycols, or mixtures of the substances listed under (a) to (i).

The tablets, coated tablets, capsules, pills and granules can be provided with the customary coatings and shells, optionally containing opacifying agents, and can also be of a composition such that they release the active compound or compounds only or preferentially in a certain part of the intestinal tract, if appropriate in a delayed manner, examples of embedding compositions which can be used being polymeric substances and waxes.

If appropriate, the active compound or compounds can also be present in microencapsulated form with one or more of the abovementioned excipients.

Formulations for parenteral administration are sterilized aqueous solutions, water-insoluble excipients, suspensions, emulsions, and suppositories.

Suppositories can contain, in addition to the active compound or compounds, the customary water-soluble or water-insoluble excipients, for example polyethylene glycols, fats, for example cacao fat, and higher esters (for example C14-alcohol with C16-fatty acid) or mixtures of these substances.

Ointments, pastes, creams and gels can contain, in addition to the active compound or compounds, the

customary excipients, for example animal and vegetable fats, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures of these substances.

Dusting powders and sprays can contain, in addition to the active compound or compounds, the customary excipients, for example lactose, talc, silicic acid, aluminum hydroxide, calcium silicate and polyamide powder, or mixtures of these substances. Sprays can additionally contain the customary propellants, for example chlorofluorohydrocarbons.

Solutions and emulsions can contain, in addition to the active compound or compounds, the customary excipients, such as solvents, solubilizing agents and emulsifiers, for example water, ethyl alcohol, isopropyl alcohol, ethylcarbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, in particular cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil and sesame oil, glycerol, glycerol formal, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, or mixtures of these substances.

For parenteral administration, the solutions and emulsions are also be in a sterile form which is isotonic with blood.

Suspensions can contain, in addition to the active compound or compounds, the customary excipients, such as liquid diluents, for example water, ethyl alcohol and propylene glycol, and suspending agents, for example ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances.

The formulation forms mentioned can also contain coloring agents, preservatives and additives which improve the smell and taste, for example peppermint oil and eucalyptus oil, and sweeteners, for example saccharin.

The abovementioned pharmaceutical formulations can also contain other pharmaceutically active compounds in addition to the compounds according to the present invention.

The abovementioned pharmaceutical formulations are prepared in the customary manner by known methods, for example by mixing the active compound or compounds with the excipient or excipients.

The effective dose of the nucleotide monomer, the antisense oligomer, or the chimeric oligomer of the present invention for use of protein synthesis inhibitors or blockers and for treatment agents of hepatitic diseases caused by virus or bacteria, cancer

or immune diseases is 0.1-50 mg/kg, and is preferably 0.2-2 mg/kg.

The oligomer of the present invention is useful for studying of proteins because it can bind to proteins as well as nucleic acids in cell. That time, the proteins contain receptors, enzymes, ligands and so on.

In addition, because it is stable to PCR and can bind to the specific sequence, the oligomer of the present invention can be used for PCR as the primer.

The oligomer of the present invention also can be used for diagnosis test using the nucleic acid hybridization as a probe (*Nucleic Acids Res.*, **1995**, <u>23</u>, 217).

Moreover, the oligomer of the present invention can be useful for the various purposes regardless of the absence or the presence of the protecting group, and can be used after the purification step. The purification process is performed by thin layer chromatography, reverse phase high-pressure liquid chromatography (HPLC), ion exchange chromatography or electrophoresis.

EXAMPLES

Practical and presently preferred embodiments of the present invention are illustrative as shown in the

following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Synthesis of the monomer nucleotide

Example 1 : Preparation of 6-N-bezoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 1, 2-isopropylidene-5-keto-α-D-glucofuranose

Dibutyltinoxide (Bu_2SnO, 48.6 g, 195 mmol) was added to 1, 2-isopropylidene-D-glucufuranose (20 g, 91 mmol) dissolved in methanol (500 mL) and was refluxed for 1 hour. After the reaction mixture was cooled at ambient temperature, the solvent was evaporated under reduced pressure. Methylene chloride (CH_2Cl_2, 500 mL) was added to the residue and this solution was cooled at 0˚C. After then, bromine (Br_2, 5.2 mL. 102 mmol) dissolved in methylene chloride (CH_2Cl_2, 100 mL) was slowly added to it. When the addition was completed, the reaction mixture was stirred at the same temperature for 10 min. The solvent was evaporated under reduced pressure. Methanol and hexane were added

little by little to the residue (solid) until two layers of the organic solution was obtained without any solid (upper layer). The hexane layer without any solid (upper layer) was removed, the methanol layer (lower layer) was concentrated under reduced pressure, the residue was purified by column chromatography (1%/ 10% $MeOH/CH_2Cl_2$) to give the desired compound (10.4 g, 53 %). The above method was referred to the reference (Baxter, E. W., Reitz, A. B. J. *Org. Chem.* (1994), vol 59, p. 3175), but it could decrease the separating time and improve the production yield by work-up after the reaction.

^1H NMR (D_2O) • 1.30 (s, 3H), 1.44 (s, 3H), 4.42 (d, 1H, J=3 Hz), 4.59 (d, 1H, J=3.2 Hz), 4.68 (m, 1H), 4.94 (d, 1H, J=3.3 Hz), 6.06 (d, 1H, J=3.5 Hz).

(Step 2) Preparation of 5-keto-D-glucose

Dowex 50WX 8-200 resin (69.83 g) was added to the title compound of the step 1 (13.65 g, 62.55 mmol) which was dissolved in distilled water (200 mL). The reaction mixture was stirred at ambient temperature for 36 hours. The resin in the reaction mixture was removed by filtration and the residual solution was freeze-dried to give the desired compound (10.3 g, 92 %).

^1H NMR (D_2O) • 3.12 (t, 1H, J=8.8 Hz), 3.40 (brt, 2H, J=9.9 Hz), 3.51 (d, 1H, J=11.8 Hz), 3.57 (t, 1H, J=10.1 Hz), 4.83 (d, 1H, J=8.2 Hz)

(Step 3) Preparation of (3S,4R,5R,6R)-2-(hydroxymethyl)-N-benzhydrylpiperidine-3,4,5-triol

The title compound (8.24 g, 46.26 mmol) prepared from the step 2 was dissolved, and the reaction mixture was added to a solution of aminodiphenylmethane (6.76 g, 36.89 mmol) and acetic acid (2.22 g, 36.97 mmol) in methanol (300 mL). Sodium hydride ($NaCNBH^3$, 5.82 g, 92.62 mmol) was added to the reaction mixture, stirred at -78°C for 2 hours, and warmed to ambient temperature. The reaction mixture was stirred at ambient temperature for 2 days, concentrated under reduced pressure. Saturated sodium bicarbonate (Na_2CO_3) solution was added. The solution was extracted with methylene chloride. The organic layer was dried by sodium sulfate (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2, 100% - 10% $MeOH/CH_2Cl_2$) to give the desired compound (5.9 g, 39%).

^1H NMR (D_2O) • 1.85 (brt, 1H, J=10.5 Hz), 2.38 (brd, 1H, J=9.2 Hz), 2.93 (dd, 1H, J=4.3, 11.3 Hz), 3.06 (brt, 1H, J=8.1 Hz), 3.51 (brm, 2H), 3.67 (brt, 1H, J=5.5 Hz), 3.83 (brd, 1H, J=4.5 Hz), 3.95 (brd, 1H,

J=10.1 Hz), 3.99 (brd, 1H, J=4.8 Hz), 4.11 (brs, 1H), 4.22 (brd, 1H, J=11.5 Hz), 5.71 (s, 1H), 7.10 - 7.24 (m, 10H).

(Step 4) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3,4-dihydroxyl-5,6-O-[(4-methoxylphenyl)ethylidene]piperidi-ne

The title compound of the step 3 (3.5 g, 1.63 mmol) was dissolved in freshly distilled methylene chloride (100 mL) and α,p-dimethoxystyrene (1.86 mL) and pyridinium p-toluenesulfonate (0.92 g, 3.67 mmol) were added to this reaction mixture. The reaction mixture was stirred at ambient temperature for 16 hours, and was extracted with the saturated sodium carbonate solution and methylene chloride. The organic layer solvent was separated, dried and concentrated under reduced pressure. The residue was purified by column chromatography (10% MeOH/CH_2Cl_2) to give the desired compound (diastereomer A:B = 1.7:1, 3.7g, 76%).

^{1}H NMR ($CDCl_3$) diastereomer A • 1.79 (s, 3H), 1.92 (ddd, 1H, J=4.8, 10.7, 10.7 Hz), 2.46 (m, 1H), 2.58 (m, 1H), 3.07 (dd, 1H, J=4.8, 11.3 Hz), 3.37 (dd, 1H, J=8.8, 9 Hz), 3.62 (dd, 1H, J=10.2, 10.5 Hz), 3.81 (s, 3H, OMe), 3.90 (dd, 1H, J=9.2, 9.2 Hz), 4.50 (dd, 1H, J=4.7, 10.8 Hz), 5.04 (s, 1H), 6.89 (d, 2H, J=8.6 Hz), 7.15 - 7.38 (m, 10H), 7.50 (d, 2H, J=8.6 Hz); diastereomer B •

1.54 (s, 3H), 1.92 (ddd, 1H, J=4.8, 10.7, 10.7 Hz), 2.46 (m, 1H), 2.89 (m, 1H), 2.99 (dd, 1H, J=4.8, 11.3 Hz), 3.37 (dd, 1H, J=8.8, 9 Hz), 3.51 (dd, 1H, J=9, 9 Hz), 3.85 (s, 3H, OMe), 3.99 (dd, 1H, J=10.5, 10.5 Hz), 4.44 (dd, 1H, J=3.9, 10.2 Hz), 4.95 (s, 1H), 6.97 (d, 2H, J=8.5 Hz), 7.15 - 7.38 (m, 12H).

(Step 5) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-hydroxyl-5,6-O-[(4-methoxylphenyl)ethylidene]piperidine

Pyridine (8.81 g, 9.01 mmol) and silver nitrate ($AgNO_3$, 5.68 g, 33.42 mmol) were added to title compound of the step 4 (13.18 g, 28.55 mmol)(or mixture of diastereomer) in tetrahydrofuran (300 mL). The reaction mixture was stirred for 20 min at ambient temperature and tert-butyldimethylsilylchloride (TBDMSiCl, 5.68 g, 37.73 mmol) was added. After stirring for 12 hrs at ambient temperature, reaction solution was filtered and the filtrate was concentrated under reduced pressure. Methylene chloride and sodium bicarbonate solution were added and shaked. The organic layer was separated, dried and concentrated under reduced pressure. The reaction mixture was purified by column chromatography (10 % ethylacetate/hexane) to give the desired compound (12 g, 73 %).

^1H NMR ($CDCl_3$) diastereomer A • 0.00 (s, 3H, Si-Me), 0.07 (s, 3H, Si-Me), 0.84 (s, 9H, Si-tBu), 1.78 (s, 3H, Me), 1.92 (dd, 1H, J=11, 11 Hz), 2.45 (ddd, 1H, J=4.4, 9.7, 9.7 Hz), 2.61 (brs, 1H, OH), 2.90 (dd, 1H, J=4.7, 9.7 Hz), 3.39 (dd, 1H, J=8.7, 8.7 Hz), 3.77 (m, 1H), 3.81 (s, 3H, OMe), 3.98 (dd, 1H, J=10.2, 10.6 Hz), 4.44 (dd, 1H, J=4.5, 10.5 Hz), 5.03 (s, 1H), 6.88 (d, 2H, J=8.8 Hz), 7.14 (d, 2H, J=8 Hz), 7.30 - 7.47 (m, 8H), 7.49 (s, 2H, J=8.8 Hz); diastereomer B • -0.07 (s, 3H, Si-Me), 0.03 (s, 3H, Si-Me), 0.82 (s, 9H, Si-tBu), 1.54 (s, 3H, Me), 1.90 (dd, 1H, J=10.5, 10.5 Hz), 2.48 (ddd, 1H, J=4, 10.4, 10.4 Hz), 2.82 (dd, 1H, J=4.8, 11.4 Hz), 3.38 (dd, 1H, J=9, 9 Hz), 3.52 (dd, 1H, J=8.9, 9.4 Hz), 3.55 (m, 1H), 3.62 (dd, 1H, J=10.5, 10.5 Hz), 3.86 (s, 3H, OMe), 4.39 (dd, 1H, J=4, 9.6 Hz), 4.94 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.15 - 7.47 (m, 12H).

(Step 6) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyoxy-4-methoxy-5,6-O-[(4-methoxyphe-nyl)ethylidene]piperidine

Iodomethane (2.74 g, 19.3 mmol, 1.2 mL) and sodium hydride (0.933 g, 233.25 mmol) were added to the title compound of the step 5, diastereomer A (5.6 g, 9.66 mmol), in anhydrous tetrahydrofuran. The reaction mixture was stirred at ambient temperature for 2 hours under nitrogen. After adding brine to the reaction mixture, it was extracted with methylene chloride,

washed the organic layer with water, dried and concentrated under reduced pressure. The residue was purified by column chromatography (10 % ethylacetate/hexane) to give the desired compound(5.38 g, 94 %).

^{1}H NMR ($CDCl_3$) diastereomer A • 0.00 (s, 3H, Si-Me), 0.06 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.75 (s, 3H, Me), 1.91 (dd, 1H, J=10.8, 10.8 Hz), 2.42 (ddd, 1H, J=3.6, 9.3, 9.3 Hz), 2.87 (dd, 1H, J=5, 11.4 Hz), 3.00 (dd, 1H, J=8.7, 8.7 Hz), 3.63 (s, 3H, OMe), 3.57 (m, 1H), 3.81 (s, 3H, OMe), 3.93 (dd, 1H, J=9, 9 Hz), 3.96 (dd, 1H, J=10, 10 Hz), 4.45 (dd, 1H, J=4.6, 10.8 Hz), 5.02 (s, 1H), 6.89 (d, 2H, J=8.9 Hz), 7.14 - 7.47 (m, 10H), 7.48 (d, 2H, J=8.8 Hz); diastereomer B • -0.07 (s, 3H, Si-Me), 0.05 (s, 3H, Si-Me), 0.82 (s, 9H, Si-tBu), 1.50 (s, 3H, Me), 1.83 (dd, 1H, J=11, 11 Hz), 2.43 (m, 1H, J=3.6, 9.3, 9.3 Hz), 2.79 (dd, 1H, J=5, 11.3 Hz), 2.97 (dd, 1H, J=8.7, 8.7 Hz), 3.52 (dd, 1H, J=9, 9 Hz), 3.53 (dd, 1H, J=10, 10 Hz), 3.67 (s, 3H, OMe), 3.77 (m, 1H), 3.86 (s, 3H, OMe), 4.38 (dd, 1H, J=4, 10.5 Hz), 4.91 (s, 1H), 6.98 (d, 2H, J=8.9 Hz), 7.14 - 7.46 (m, 12H).

(Step 7) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-hydroxyl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene] piperidene

Tetrabutylammonium fluoride (1 M solution dissolved in tetrahydrofuran, 25 mL, 25 mmol) was added to the title compound of step 6 in tetrahydrofuran. After stirring at ambient temperature for 2.5 hrs, the reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography (10 - 40 % ethylacetate/hexane) to give the desired compound (3.44 g, 86 %).

^1H NMR ($CDCl_3$) diastereomer A • 1.77 (s, 3H, Me), 1.93 (dd, 1H, J=10.8, 10.8 Hz), 2.47 (ddd, 1H, J=4.7, 10.1, 10.1 Hz), 3.02 (dd, 1H, J=9, 9 Hz), 3.08 (dd, 1H, J=5, 11.2 Hz), 3.71 (s, 3H, OMe), 3.82 (s, 3H, OMe), 4.00 (dd, 1H, J=10.5, 10.5 Hz), 4.03 (dd, 1H, J=9, 9 Hz), 4.51 (dd, 1H, J=4.5, 10.8 Hz), 5.05 (s, 1H), 6.89 (d, 2H, J=8.8 Hz), 7.14 - 7.47 (m, 10H), 7.47 (d, 2H, J=8.8 Hz); diastereomer B • 1.58 (s, 3H, Me), 1.91 (dd, 1H, J=10.5, 10.5 Hz), 2.48 (ddd, 1H, J=4, 10.3, 10.3 Hz), 3.02 - 2.98 (m, 2H), 3.70 - 3.57 (m, 3H), 3.82 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.45 (dd, 1H, J=4, 10.5 Hz), 4.95 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.16 - 7.39 (m, 12H).

(Step 8) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-methanesulfonyl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethyl-idene]piperidine

Methanesulfonyl chloride (2.15 g, 18.81 mmol) and

triethylamine (1.46 mL) were added to the title compound of the step 7 (2.98 g, 6.27 mmol) which was dissolved in distilled methylene chloride (100 mL), and was stirred for 1 hours. The reaction mixture was extracted with sodium bicarbonate solution and methylene chloride. The organic layer was separated, dried and concentrated under reduced pressure. The residue was purified by column chromatography (20 % - 50 % ethlyacetate/hexane) to give the desired compound (3.44 g, 99 %).

^{1}H NMR ($CDCl_3$) diastereomer A • 1.77 (s, 3H, Me), 2.07 (dd, 1H, J=11, 11 Hz), 2.44 (ddd, 1H, J=4.7, 9.9, 9.9 Hz), 3.09 (s, 3H, OMs), 3.21 (m, 1H), 3.22 (dd, 1H, J=9, 9 Hz), 3.67 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.99 (dd, 1H, J=10.6, 10.6 Hz), 4.03 (dd, 1H, J=9.1, 9.1 Hz), 4.52 (dd, 1H, J=4.5, 10.9 Hz), 4.59 (ddd, 1H, J=5.2, 10.5, 10.5 Hz), 5.07 (s, 1H), 6.89 (d, 2H, J=8.9 Hz), 7.15 - 7.38 (m, 10H), 7.46 (d, 2H, J=8.9 Hz); diastereomer B • 1.53 (s, 3H, Me), 2.04 (dd, 1H, J=10.9, 10.9 Hz), 2.45 (ddd, 1H, J=4, 10.2, 10.2 Hz), 3.07 (s, 3H, OMs), 3.19 (dd, 1H, J=9.2, 9.2 Hz), 3.60 (dd, 1H, J=4, 10.4 Hz), 3.62 (dd, 1H, J=9.1, 9.1 Hz), 3.73 (s, 3H, OMe), 3.87 (s, 3H, OMe), 4.40 (dd, 1H, J=5.2, 9.3 Hz), 4. 43 (ddd, 1H, J=4.2, 10.8, 10.8 Hz), 4.96 (s, 1H), 6.99 (d, 2H, J=8.8 Hz), 7.14 - 7.39 (m, 12H).

(Step 9) Preparation of {(3R,4R,5R,6R)-N-banzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

Adenine (0.82 g, 6.07 mmol), sodium hydride (304 mg, 7.6 mmol) and 18-crown-6 (319 mg, 1.21 mmol) were dissolved in anhydrous N,N-dimethylformamide (75 mL) and stirred at 80°C for 1 hour. The title compound of the step 8, diastereomer B (1.67 g, 3.02 mmol)(diastereomer A or A and B mixture) dissolved in anhydrous N,N-dimethylformamide (25 mL) was added to the reaction mixture, heated at 100°C and stirred for 16 hours. After cooling and concentrating the reaction mixture under reduced pressure, it was dissolved in ethylacetate (400 mL) and washed with the saturated sodium bicarbonate solution (25 mL) and water (50 mL). After evaporating the organic solvent under reduced pressure, the residue was purified by column chromatography (5 % methanol/methylene chloride) to dive the desired compound (900 mg, 50 %).

^{1}H NMR ($CDCl_3$) • 1.55 (s, 3H), 2.74 (m, 2H), 2.80 (dd, 1H, J=11.3, 11.5 Hz), 2.98 (m, 1H), 3.37 (s, 3H, OMe), 3.69 (dd, 1H, J=10.4, 10.4 Hz), 3.92 (m, 2H), 3.93 (s, 3H, OMe), 4.47, (ddd, 1H, J=4, 10.6, 10.6 Hz), 4.48 (m, 1H), 5.02 (s, 1H), 6.99 (d, 2H, J=8.8 Hz)), 7.13 - 7.42 (m, 12H), 7.71 (s, 1H), 8.32 (s, 1H).

(Step 10) Preparation of 6-N-benzoly-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidinr-3-yl}adenine

The title compound of the step 9 (1.65 g, 2.78 mmol) was dissolved in pyridine (100 mL) and cooled at 0˚C. After dropping benzoyl chloride (1.17 g, 8.34 mmol) to the reaction mixture for 30 min, it was stirred for 2 hours. The reaction mixture was cooled at 0•C , added with water (2.7 mL) and stirred for 5 min. After adding ammonia water(5.56 mL) to the reaction mixture at ambient temperature, it was stirred for 15 min, added with water (500 mL) and extracted with methylene chloride (200 mL). The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (10 % methanol/methylenechloride) to give the desired compound (1.27 g, 66 %).

^{1}H NMR ($CDCl_3$) • 1.55 (s, 3H), 2.75 (ddd, 1H, J=3, 9.1, 9.1 Hz), 2.82 (dd, 1H, J=11.4, 11.4 Hz), 3.05 (dd, J=4.5, 11.3 Hz), 3.39 (s, 3H, OMe), 3.71 (dd, 1H, J=10.5, 10.5 Hz), 3.82 (dd, 1H, J=9, 9 Hz), 3.88 (s, 3H, OMe), 3.96 (dd, 1H, J=9.1, 10 Hz), 4.49 (m, 2H), 5.03 (s, 1H), 7.00 (d, 2H, J=8.7 Hz), 7.13 - 7.41 (m, 12H), 7.52 (t, 2H, J=6.7 Hz), 7.60 (d, 1H, J=7.1 Hz), 7.91 (s, 1H), 8.02 (d, 2H, J=7.3 Hz), 8.75 (s, 1H).

(Step 11) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidi-ne-3-yl}adenine

The title compound of the step 10 (1.22 g, 1.75 mmol) was dissolved in 80 % acetic acid (10 g) and stirred at 45 - 50•C for 3 hours. After adding methylene chloride (100 ml) and the saturated sodium bicarbonate solution (20 mL) to the reaction mixture. The organic layer was separated, dried and concentrated under reduced pressure. The residue was purified by column chromatography (5 % methanol/methylene chloride) to give the desired compound (543 mg, 55 %).

^{1}H NMR ($CDCl_3$) • 2.74 (m, 1H), 2.97 (dd, 1H, J=11, 11 Hz), 3.10 (s, 3H, OMe), 3.19 (dd, 1H, J=4, 12 Hz) 3.78 (dd, 1H, J=8.5, 9.7 Hz), 4.02 (dd, 1H, J=8.5, 8.5 Hz), 4.17 (dd, 1H, J=2, 12 Hz), 4.27 (dd, 1H, J=3.5, 12 Hz), 4.61 (ddd, 1H, J=4.2, 10.5, 10.5 Hz), 5.55 (s, 1H), 7.19 - 7.53 (m, 10H), 7.55 (t, 2H, J=7 Hz), 7.61 (d, 1H, J=7 Hz), 8.02 (s, 1H), 8.04 (d, 2H, J=7.2 Hz), 8.76 (s, 1H).

(Step 12) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-metho-xypiperidine-3-yl}adenine

4,4'-Dimethoxytrityl chloride (0.752 g, 2.22 mmol) was added to the title compound of the step 11

(500 mg, 0.89 mmol) which was dissolved in pyridine (10 mL) at 0•C . The reaction mixture was stirred for 16 hours, and concentrated under reduced pressure. 5 % Sodium bicarbonate (10 mL) solution was added to the reaction mixture. The reaction mixture was extracted with methylene chloride (100 mL). The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:Hexane:TEA = 50:50:1) to give the desired compound (672 mg, 88 %).

^{1}H NMR ($CDCl_3$) • 2.68 (m, 1H), 2.78 (m, 1H), 2.97 (dd, 1H, J=11, 11 Hz), 3.09 (s, 3H, OMe), 3.12 (dd, 1H, J=4.2, 11.8 Hz) 3.65 (dd, 1H, J=3.2, 10.4 Hz), 3.793 (s, 3H, OMe), 3.796 (s, 3H, OMe), 4.21 (dd, 1H, J=8.5, 8.5 Hz), 4.63 (ddd, 1H, J=4.2, 10.3, 10.3 Hz), 5.09 (s, 1H), 6.81 (d, 2H, J=8.7 Hz), 6.82 (d, 2H, J=9 Hz), 7.18 - 7.32 (m, 16H), 7.37 (d, 2H, J=8.9 Hz), 7.38 (d, 2H, J=8.9 Hz), 7.51 (t, 2H, J=6.8 Hz), 7.59 (d, 1H, J=7.2 Hz), 8.01 (s, 1H), 8.02 (d, 2H, J=7 Hz), 8.78 (s, 1H).

(Step 13) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypipe ridine-3-yl}adenine

After evaporating the title compound of the step 12 (654 mg, 0.754 mmol) with anhydrous toluene three

times using vacuum pump, it was dissolved in methylene chloride(7 mL) under nitrogen, and N,N-diisopropylethylamine (356 μl) was added to the reaction mixture. 2-Cyanoethyl N,N-diisopropyl chloro phosphoramidite (356 μl) was added to the reaction mixture, and it was stirred for 5 hours under nitrogen. The reaction solution was washed with 5 % sodium bicarbonate solution (10 mL) and extracted with methylene chloride (100 mL). The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:Hexane:TEA = 50:50:1) to give the desired compound (diastereomer mixture 465 mg, 58 %).

^{1}H NMR ($CDCl_3$) • 1.04 (d, 6H, J=6.7 Hz), 1.15 (d, 6H, J=6.5 Hz), 1.19 (d, 6H, J=6.5 Hz), 1.21 (d, 6H, J=6.7 Hz), 2.52 (t, 4H, J=6 Hz), 2.63 (d, 1H, J= 11.1 Hz), 2.73 (dd, 1H, J=2, 13 Hz), 2.84 (dd, 1H, J=4, 6 Hz), 2.92 (dd, 1H, J=3.5, 13.5 Hz), 3.27 (s, 3H, OMe), 3.33 (s, 3H, OMe), 3.36 - 3.80 (m, 11H), 3.834 (s, 6H, OMe), 3.838 (s, 6H, OMe), 3.84 (m, 2H), 4.34 (d, 2H, J=11.1 Hz), 4.40 (d, 2H, J=10.6 Hz), 4.68 (s, 1H), 4.70 (s, 1H), 4.77 (m, 2H), 6.83 (d, 8H, J=8.8 Hz), 7.12 - 7.39 (m, 30H), 7.55 (t 4H, J=7 Hz), 7.62 (d, 2H, J=7.2 Hz), 8.07 (d, 4H, J=7.3 Hz), 8.72 (s, 1H), 8.75 (s, 1H), 9.06 (s, 1H), 9.32 (s, 1H).

^{31}P NMR ($CDCl_3$) • 149.01, 150.07.

Example 2 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine

(Step 1) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

This compound was prepared from the diastereomer A (diastereomer B or A and B mixture) of the Example 1 (step 5) as a starting material via the procedure described in the Example 1 using iodoethane (EtI).

^{1}H NMR ($CDCl_3$) • -0.02 (s, 3H, Si-Me), 0.07 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.24 (t, 3H, J=7.1 Hz, $OCH_2\underline{CH_3}$), 1.75 (s, 3H, Me), 1.91 (dd, 1H, J=10.8, 10.8 Hz), 2.43 (ddd, 1H, J=4.6, 9.4, 9.4 Hz), 2.88 (dd, 1H, J=4.8, 11.5 Hz), 3.08 (dd, 1H, J=8.7, 8.7 Hz), 3.74 (m, 1H), 3.82 (s, 3H, OMe), 3.94 (m, 4H), 4.45 (dd, 1H, J=4.2, 10.3 Hz), 5.03 (s, 1H), 6.88 (d, 2H, J=8.9 Hz), 7.14 - 7.40 (m, 10H), 7.46 (d, 2H, J=8.9 Hz).

(Step 2) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-hydroxyl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene] piperidine

This compound was prepared from the title

compound of the step 1 via the procedure described in the step 7 of the Example 1.

^1H NMR ($CDCl_3$) • 1.26 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 1.76 (s, 3H, Me), 1.93 (dd, 1H, J=10.8, 10.8 Hz), 2.45 (ddd, 1H, J=4.4, 9.7, 10.1 Hz), 3.11 (m, 2H), 3.71 (m, 1H), 3.82 (s, 3H, OMe), 3.99 (m, 1H), 4.03 (dd, 1H, J=4.2, 9 Hz), 4.12 (q, 2H, J=7 Hz), 4.51 (dd, 1H, J=4, 6 Hz), 5.05 (s, 1H), 6.90 (d, 2H, J=9 Hz), 7.14 - 7.36 (m, 10H), 7.45 (d, 2H, J=9 Hz).

(Step 3) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-methanesulfonyl-4-ethoxy-5,6-O-[(4methoxyphenyl) ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 8 of the Example 1.

^1H NMR ($CDCl_3$) • 1.24 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 1.77 (s, 3H, Me), 2.07 (dd, 1H, J=11.1, 11.1 Hz), 2.43 (ddd, 1H, J=4.5, 9.7, 9.7 Hz), 3.11 (s, 3H, OMs), 3.25 (dd, 1H, J=5.3, 11.3 Hz), 3.32 (dd, 1H, J=9, 9 Hz), 3.71 (q, 2H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.82 (s, 3H, OMe), 4.03 (m, 2H), 4.03 (dd, 1H, J=9, 9 Hz), 4.52 (dd, 1H, J=4.4, 11 Hz), 4.58 (ddd, 1H, J=5.2, 9.8, 9.8 Hz), 5.07 (s, 1H), 6.89 (d, 2H, J=8.8 Hz), 7.13 - 7.37 (m, 10H), 7.44 (d, 2H, J=8.8 Hz).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) • 0.88 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 1.80 (s, 3H, Me), 2.71 (ddd, 1H, J=4.4, 9.8, 9.8 Hz), 2.90 (dd, 1H, J=11.3, 11.3 Hz), 3.10 (dd, 1H, J=4.7, 11.1 Hz), 3.23 (q, 1H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.76 (q, 1H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.82 (s, 3H, OMe), 3.95 (dd, 1H, J=9.9, 9.9 Hz), 4.07 (dd, 1H, J=10.6, 10.6 Hz), 4.20 (dd, 1H, J=8.9, 8.9 Hz), 4.53 (dd, 1H, J=4.5, 11 Hz), 4.64 (ddd, 1H, J=4.5, 10.8, 10.8 Hz), 5.13 (s, 1H), 6.88 (d, 2H, J=8.8 Hz), 7.14 - 7.47 (m, 12H), 7.73 (s, 1H), 8.31 (s, 1H).

(Step 5) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene] piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) • 0.79 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$),

1.81 (s, 3H, Me), 2.73 (ddd, 1H, J=4.4, 9.7, 9.7 Hz), 2.93 (dd, 1H, J=11.5, 11.5 Hz), 3.15 (dd, 1H, J=4.4, 11.3 Hz), 3.22 (q, 1H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.80 (q, 1H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.82 (s, 3H, OMe), 3.98 (dd, 1H, J=9.1, 9.1 Hz), 4.08 (dd, 1H, J=10.6, 10.6 Hz), 4.23 (dd, 1H, J=8.9, 8.9 Hz), 4.54 (dd, 1H, J=4.5, 10.9 Hz), 4.72 (ddd, 1H, J=4.6, 8.8, 9.9 Hz), 5.14 (s, 1H), 6.88 (d, 2H, J=8.8 Hz), 7.14 - 7.56 (m, 15H), 7.95 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.76 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-ethoxypiperidine -3-yl}adenine

This compound was prepared from the title compound of the step 11 via the procedure described in the step 5 of the Example 1.

^1H NMR ($CDCl_3$) • 0.85 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 2.73 (m, 1H), 2.98 (m, 2H), 3.05 (d, 1H, J=11.2 Hz), 3.20 (dd, 1H, J=4.1, 11.6 Hz), 3.37 (dq, 1H, J=2.3, 7 Hz, $OCH_2C\underline{H}_3$), 3.81 (dd, 1H, J=9.5, 9.5 Hz), 4.01 (dd, 1H, J=8.4, 8.4 Hz), 4.28 (dd, 1H, J=3.3, 12.1 Hz), 4.59 (ddd, 1H, J=4, 10.3, 10.3 Hz), 5.56 (s, 1H), 7.20 - 7.64 (m, 13H), 8.01 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.76 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-

benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-ethoxy piperidine-3-yl}denine

This compound was prepared from the title compound of the step 12 via the procedure described in the step 6 of the Example 1.

^1H NMR ($CDCl_3$) • 0.84 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 2.51 (d, 1H, J=3 Hz), 2.64 (d, 1H, J=8.5 Hz), 2.91 (dq, 1H, J=2.3, 7 Hz), 3.06 (dd, 1H, J=10.9 Hz), 3.15 (dd, 1H, J=4.6, 11.4 Hz), 3.41 (dq, 1H, J=2.3, 7 Hz, $OCH_2C\underline{H}_3$), 3.66 (dd, 1H, J=3.2, 10.4 Hz), 3.79 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.81 (m, 1H), 4.22 (dd, 1H, J=2.5, 7.4 Hz), 4.62 (ddd, 1H, J=4.4, 10.4, 10.4 Hz), 5.09 (s, 1H), 6.82 (d, 2H, J=9 Hz), 6.83 (d, 2H, J=9 Hz), 7.18 - 7.65 (m, 22H), 8.01 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.79 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperi-dine-3-yl}adenine

This compound was prepared from the title compound of the step 13 via the procedure described in the step 7 of the Example 1.

^1H NMR ($CDCl_3$) • 1.01 (t, 3H, J=6.8 Hz, $OCH_2C\underline{H}_3$), 1.03 (d, 6H, J=6.7 Hz), 1.13 (d, 6H, J=6.7 Hz), 1.16 (d,

6H, J=6.7 Hz), 1.20 (d, 6H, J=6.7 Hz), 2.52 (m, 4H), 2.60 (d, 2H, J=11.1 Hz), 2.78 (dd, 2H, 3.7, 11.5 Hz), 2.94 (dd, 2H, J=3.6, 11.4 Hz), 3.01 (dd, 2H, J=4.7, 11.1 Hz), 3.18 - 3.80 (m, 16H), 3.83 (s, 6H, OMe), 3.84 (s, 6H, OMe), 4.28 (d, 2H, J=13.6 Hz), 4.41 (d, 2H, J=13.5 Hz), 4.72 (m, 2H), 4.76 (s, 2H), 6.82 (d, 8H, J=8.7 Hz), 7.08 - 7.66 (m, 44H), 8.07 (d, 4H, J=7.2 Hz), 8.73 (s, 1H), 8.75 (s, 1H), 9.08 (s, 1H), 9.34 (s, 1H).

^{31}P NMR ($CDCl_3$) µl 148.85, 149.96.

Example 3 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxyethoxy piperidine-3-yl}adenine

(Step 1) Preparation of (3S,4R,5R,6R)-N-banzhydryl-3-tertbutyldimethylsilyloxy-4-ethoxymethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

The diastereomer B (diastereomer A or A and B mixture)(200 mg, 0.61 mmol) obtained from the step 5 of the Example 1 was dissolved in anhydrous tetrahydrofuran (5 mL), and sodium hydride (72 mg, 1.8 mmol) in tetrahydrofuran (2 mL) was added to the reaction mixture. After heating the reaction mixture at 60•C , 2-bromoethylmethylether (171 •L, 1.8 mmol) was added to the reaction solution, and it was stirred at 60•C for 1 day. After adding water, the reaction

mixture was extracted with ethylacetate, dried by sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 5 - 10% ethylacetate/hexane solvent to give the desired compound (215 mg, 56 %).

^1H NMR ($CDCl_3$) • 0.13 (s, 3H, Si-Me), 0.20 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.51 (s, 3H, Me), 1.83 (dd, 1H, J=11, 11 Hz), 2.41 (m, 1H), 2.87 (dd, 1H, J=5, 11.4 Hz), 3.03 (dd, 1H, J=4.7, 11.5 Hz), 3.23 (s, 3H, OMe), 3.26 - 3.61 (m, 4H), 3.73 (ddd, 1H, J=5, 8.7, 8.7 Hz), 3.85 (s, 3H, OMe), 3.92 (d, 1H, J=9 Hz), 3.98 (d, 1H, J=10.7 Hz), 4.42 (m, 1H), 4.95 (s, 1H), 6.93 (d, 2H, J=8.8 Hz), 7.16 - 7.40 (m, 12H).

(Step 2) Preparation of (3S,4R,5R,6R)-N-benahydryl-3-hydroxyl-4-methoxyethoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 7 of the Example 1.

^1H NMR ($CDCl_3$) • 1.51 (s, 3H, Me), 1.88 (dd, 1H, J=10.8, 10.8 Hz), 2.45 (ddd, 1H, J=2.8, 9.2, 9.2 Hz), 2.76 (m, 1H), 3.02 (dd, 1H, J=5, 11.8 Hz), 3.11 (dd, 1H, J=9, 9 Hz), 3.43 (s, 3H, OMe), 3.55 - 3.68 (m, 4H), 3.86 (s, 3H, OMe), 3.90 (m, 1H), 4.26 (ddd, 1H, J=3,

5.4, 11.8 Hz), 4.44 (dd, 1H, J=4, 11 Hz), 4.93 (s, 1H), 6.97 (d, 2H, J=8.7 Hz), 7.20 - 7.40 (m, 12H).

(Step 3) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-methanesulfonyl-4-methoxyethoxy-5,6-O-[(4methoxyphenyl) ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.52 (s, 3H, Me), 2.04 (dd, 1H, J=11, 11 Hz), 2.46 (ddd, 1H, J=4, 10, 10 Hz), 3.15 (s, 3H, OMs), 3.33 (d, 1H, J=11 Hz), 3.41 (s, 3H, OMe), 3.61 - 3.68 (m, 2H), 3.86 (s, 3H, OMe), 3.88 (m, 1H), 4.18 (dd, 1H, J=3, 5.7 Hz), 4.22 (dd, 1H, J=3, 5.7 Hz), 4.39 (dd, 1H, J=4.5, 9.5 Hz), 4.45 (dd, 1H, J=4, 10 Hz), 4.95 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.14 - 7.40 (m, 12H).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-banzhydryl-4-methoxyethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.54 (s, 3H, Me), 2.75 (ddd, 1H,

J=4, 10, 10 Hz), 2.95 (m, 1H), 3.12 (s, 3H, OMe), 3.14 - 3.22 (m, 2H), 3.48 (ddd, 1H, J=3, 5.8, 5.8), 3.81 (dd, 1H, J=6.2, 6.2 Hz),3.85 (m, 1H), 3.87 (s, 3H, OMe), 3.94 (ddd, 1H, J=3.2, 6, 11.4 Hz), 4.13 (dd, 1H, J=9.5, 9.5 Hz), 4.42 (dd, 1H, J=5, 10.2 Hz), 4.47 (dd, 1H, J=4, 10.5 Hz), 5.00 (s, 1H), 6.98 (d, 2H, J=8.7 Hz), 7.14 - 7.44 (m, 12H), 7.74 (s, 1H), 8.30 (s, 1H).

(Step 5) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxyethoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.55 (s, 3H, Me), 2.74 (ddd, 1H, J=5, 9, 9 Hz), 2.97 (dd, 1H, J=11.2, 11.2 Hz), 3.03 (m, 1H), 3.11 (s, 3H, OMe), 3.14 - 3.21 (m, 2H), 3.46 (ddd, 1H, J=3.1, 6, 11.5), 3.70 (dd, 1H, J=10.5, 10.5 Hz), 3.83 (d, 1H, J=8.9 Hz), 3.88 (s, 3H, OMe), 3.98 (ddd, 1H, J=3, 5.5, 5.5 Hz), 4.17 (dd, 1H, J=9.2, 9.2 Hz), 4.51 (m, 1H), 4.54 (ddd, 1H, J=5, 11, 11 Hz), 5.02 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.14 - 7.62 (m, 12H), 7.96 (s, 1H), 8.03 (d, 2H, J=7.4 Hz), 8.76 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-methoxyethoxypi

-peridine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) • 2.69 (m, 1H), 2.90 (dd, 1H, J=11.5, 11.5), 3.17 (dd, 1H, J=4, 11.5 Hz), 3.31 (s, 3H, OMe), 3.24 - 3.40 (m, 4H), 3.89 (dd, 1H, J=9, 9 Hz), 4.01 (dd, 1H, J=8.7, 8.7 Hz), 4.23 (m, 2H), 4.57 (ddd, 1H, J=4, 10.7, 10.7 Hz), 5.59 (s, 1H), 7.17 - 7.38 (m, 10H), 7.51 (t, 2H, J=7.2 Hz), 7.59 (d, 1H, J=7.3 Hz), 7.90 (s, 1H), 8.05 (d, 2H, J=7.2 Hz), 8.72 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxyethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) • 2.60 (m, 1H), 3.02 (m, 1H), 3.16 (dd, 1H, J=4.3, 11.3), 3.36 (s, 3H, OMe), 3.26 - 3.38 (m, 4H), 3.57 (d, 1H, J=9.5 Hz), 3.79 (s, 3H, OMe), 3.86 (d, 1H, J=9.8 Hz), 3.95 (dd, 1H, J=10, 10 Hz), 4.33 (dd, 1H, J=8.8, 8.8 Hz), 4.63 (ddd, 1H, J=4.2, 10.8, 10.8 Hz), 5.02 (s, 1H), 6.79 (d, 2H, J=8.8 Hz), 6.81 (d, 2H, J=8.8 Hz), 7.22 - 7.42 (m, 12H), 7.53 (t,

2H, J=7.3 Hz), 7.61 (d, 1H, J=7.3 Hz), 7.97 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.78 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxyethoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.01 (d, 6H, J=6.8 Hz), 1.15 (d, 6H, J=6.8 Hz), 1.19 (d, 6H, J=6.8 Hz), 1.24 (d, 6H, J=6.8 Hz), 2.49 (m, 4H), 2.60 (m, 2H), 2.77 (m, 3H), 2.94 (m, 2H), 3.21 (s, 3H, OMe), 3.23 (s, 3H, OMe), 3.27 - 3.59 (m, 18H), 3.83 (s, 12H, OMe), 4.17 (m, 1H), 4.28 (d, 2H, J=13.4 Hz), 4.40 (d, 2H, J=12.3 Hz), 4.75 (s, 2H), 4.77 (m, 2H), 6.81 (d, 8H, J=8.4 Hz), 7.06 - 7.35 (m, 38H), 7.53 (d, 4H, J=7.5 Hz), 7.61 (d, 2H, J=7.5 Hz), 8.05 (d, 4H, J=7.3 Hz), 8.75 (s, 1H), 9.02 (s, 1H), 9.13 (s, 1H), 9.29 (s, 1H).

^{31}P NMR ($CDCl_3$) •: 148.99, 149.77.

Example 4 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethylpiperidine-3-yl} adenine

(Step 1) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-(imidazole-1-yl)-thiocarboxyl-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

1,1'-Thiocarbonyldiimidazole (12.44 g, 69.81 mmol) was added to the title compound, diastereomer A (12.0 g, 20.84 mmol)(diastereomer B or A and B mixture) obtained from the step 6 of the example 1 which was dissolved in acetonitrile (200 mL). The reaction mixture was refluxed for 24 hours and concentrated under reduced pressure. Methylene chloride and water were added to the reaction mixture, and the organic layer was extracted. The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:Hexane = 1:4) to give the desired compound (3.33 g, 23 %).

^{1}H NMR ($CDCl_3$) • -0.18 (s, 6H, Si-Me), 0.68 (s, 9H, Si-tBu), 1.45 (s, 3H, Me), 2.12 (dd, 1H, J=11, 11 Hz), 2.65 (ddd, 1H, J=4, 10.1, 10.1 Hz), 2.94 (dd, 1H, J=5, 11.6 Hz), 3.70 (dd, 1H, J=10.6, 10.6 Hz), 3.74 (m, 1H), 3.43 (s, 1H, OMe), 3.44 (m, 1H), 4.99 (s, 1H), 5,78 (dd, 1H, J=9, 9 Hz), 6.93 (d, 2H, J=8.8 Hz), 7.16 - 7.43 (m, 12H), 7.76 (s, 1H), 8.46 (s, 1H).

(Step 2) Preparation of (3S,5R,6R)-N-benzhydryl-3-

tertbutyldimethylsilyloxy-5,6-O-[(4-methoxyphenyl)ethyl-idene]piperidine

Tributyltin hydride (2.22g 7.62 mmol) and 2,2'-azobis(isobutyronitryl)(0.82 g, 4.97 mmol) was added to the title compound of the step 1 (2.62g 3.82 mmol) which was dissolved in toluene (30 mL) and stirred for 2 hours. The reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (10% - 20% EtOAc/Hexane) to give the desired compound (2.0 g, 47 %).

^{1}H NMR ($CDCl_3$) • -0.03 (s, 3H, Si-Me), 0.00 (s, 3H, Si-Me), 0.82 (s, 9H, Si-tBu), 1.55 (s, 3H, Me), 1.81 (dd, 1H, J=10.5, 10.5 Hz), 2.18 (m, 1H), 2.33 (ddd, 1H, J=4, 10.5, 10.5 Hz), 2.86 (dd, 1H, J=4, 10.3 Hz), 3.49 (ddd, 1H, J=4.4, 10.5, 10.5 Hz), 3.61 (dd, 1H, J=10.5, 10.5 Hz), 3.69 (m, 1H), 3.86 (s, 3H, OMe), 4.50 (dd, 1H, J=4, 10.5 Hz), 4.95 (s, 1H), 6.97 (d, 2H, J=8.8 Hz), 7.16 - 7.47 (m, 12H).

(Step 3) Preparation of (3S,5R,6R)-N-benzhydryl-3-hydroxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.52 (s, 3H, Me), 1.76 (dd, 1H,

J=10.5, 10.5 Hz), 2.32 (m, 2H), 3.00 (dd, 1H, J=3, 10.5 Hz), 3.51 - 3.59 (m, 2H), 3.66 (m, 1H), 3.76 (m, 1H), 3.86 (s, 3H, OMe), 4.47 (dd, 1H, J=4, 10.5 Hz), 4.97 (s, 1H), 6.96 (d, 2H, J=6.8 Hz), 7.17 - 7.40 (m, 12H).

(Step 4) Preparation of (3S,5R,6R)-N-benzhydryl-3-methanesulfoxy-5,6-O-[(4-methoxyphenyl)ethylidene]pipe-ridine

This compound was prepared via the procedure described in the step 8 of the Example.

^{1}H NMR ($CDCl_3$) • 1.52 (s, 3H, Me), 2.01 (dd, 1H, J=10.5, 10.5 Hz), 2.38 (ddd, 1H, J=4, 10.2, 10.2 Hz), 2.53 (m 1H), 2.95 (s, 3H, OMs), 3.14 (dd, 1H, J=4, 11.5 Hz), 3.56 (m, 1H), 3.63 (dd, 1H, J=10.5, 10.5 Hz), 3.87 (s, 3H, OMe), 4.46 (dd, 1H, J=4, 10.5 Hz), 4.67 (m, 1H), 4.98 (s, 1H), 6.96 (d, 2H, J=8.6 Hz), 7.19 - 7.42 (m, 12H).

(Step 5) Preparation of {(3R,5R,6R)-N-benzhydryl-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.54 (s, 3H, Me), 2.24 (dd, 1H, J=10.8, 10.8 Hz), 2.55 (m, 2H), 3.20 (m, 1H), 3.72 (dd,

1H, J=10.5, 10.5 Hz), 3.86 (m 1H), 3.88 (s, 3H, OMe), 4.53 (dd, 1H, J=4, 10.5 Hz), 4.77 (m, 1H), 5.06 (s, 1H), 6.98 (d, 2H, J=8.7 Hz), 7.12 - 7.39 (m, 12H), 8.03 (s, 1H), 8.32 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,5R,6R)-N-benzhydryl-5,6-O-[(4-methoxyohenyl)ethylidene] piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.55 (s, 3H, Me), 2.27 (dd, 1H, J=11, 11 Hz), 2.55 (m, 2H), 3.21 (m, 1H), 3.74 (dd, 1H, J=10.5, 10.5 Hz), 3.78 (m, 1H), 3.88 (s, 3H, OMe), 4.54 (dd, 1H, J=4, 10.5 Hz), 4.77 (m, 1H), 5.07 (s, 1H), 6.99 (d, 2H, J=8.7 Hz), 7.14 - 7.55 (m, 15H), 7.94 (s, 1H), 8.03 (d, 2H, J=7.2 Hz), 8.75 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethylpiperidine-3-yl} adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) • 2.07 (m, 1H), 2.54 (m, 2H), 2.68

(m, 1H), 3.25 (dd, 1H, J=3, 11.3 Hz), 4.17 - 4.24 (m, 3H), 4.85 (m, 1H), 5.43 (s, 1H), 7.18 - 7.33 (m, 10H), 7.52 (t, 2H, J=7 Hz), 7.61 (d, 1H, J=7.3 Hz), 8.05 (d, 2H, J=7.2 Hz), 8.26 (s, 1H), 8.70 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethylpiperidi-ne-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) • 2.45 (m, 2H), 2.63 (dd, 1H, J=7, 12.4 Hz), 2.85 (m, 1H), 3.14 (dd, 1H, J=3, 12, 12 Hz), 3.52 (dd, 1H, J=6, 10 Hz), 3.74 (dd, 1H, J=3, 10 Hz), 3.823 (s, 3H, OMe), 3.829 (s, 3H, OMe), 4.25 (m, 1H), 4.84 (m, 1H), 4.92 (s, 1H), 6.85 (d, 2H, J=8.9 Hz), 6.87 (d, 2H, J=8.9 Hz), 7.15 - 7.35 (m, 12H), 7.44 (d, 2H, J=7 Hz), 7.55 (t, 2H, J=7.3 Hz), 7.61 (d, 1H, J=7.3 Hz), 8.05 (d, 2H, J=7 Hz), 8.52 (s, 1H), 8.78 (s, 1H).

(Step 9) Preparation of 6-N-benzoyl-{(3R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethylpiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 8 via the procedure described in

the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.05 (d, 6H, J=6.7 Hz), 1.10 (d, 6H, J=6.7 Hz), 1.16 (d, 6H, J=6.7 Hz), 1.19 (d, 6H, J=6.7 Hz), 2.28 (m, 2H), 2.73 (m, 2H), 2.77 (m, 4H), 2.96 (dd, 2H, J=2, 12.8 Hz), 3.10 (dd, 2H, J=3, 13 Hz), 3.33 (dd, 2H, J=8.2, 8.7 Hz), 3.42 - 3.75 (m, 12H), 3.82 (s, 3H, OMe), 3.83 (s, 6H, OMe), 3.84 (s, 3H, OMe), 4.37 (m, 1H), 4.57 (s, 1H), 4.60 (s, 1H), 4.91 (m, 2H), 6.85 (d, 4H, J=7.2 Hz), 6.86 (d, 4H, J=7.2 Hz), 7.07 - 7.42 (m, 34H), 7.55 (d, 4H, J=7.7 Hz), 7.62 (d, 2H, J=7.2 Hz), 8.09 (d, 4H, J=7.3 Hz), 8.71 (s, 1H), 8.72 (s, 1H), 9.17 (s, 1H), 9.30 (s, 1H).

^{31}P NMR ($CDCl_3$) •: 148.30, 148.59

Example 5 : Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypipe ridine-3-yl}adenine

(Step 1) Preparation of (3S,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-oxo-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

After adding dimethylsulfoxide (253 •, 3.81 mmol) and oxalyl chloride (1.8 mL, mmol) to anhydrous dichloromethane (40 mL) at -78 •C , the title compound, diastereomer B (1 g, 1.73 mmol)(diastereomer A or A and

B mixture) obtained from the step 5 in the Example 1 was added to it for 5 min. After stirring for 15 min, triethylamine (574 •, 0.87 mmol) was added to it at -78 •C . A cooling device was removed after 5 min, and the temperature of reaction mixture was allowed to reach ambient temperature. Distilled water was added to the reaction mixture, the organic solvent layer was separated, dried by sodium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography (5~10 %) eluted with methanol/dichloromethane solvent system to give the desired compound (700 mg, 71 %).

^{1}H NMR ($CDCl_3$) • -0.15 (s, 3H, Si-Me), 0.08 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.59 (s, 3H, Me), 2.30 (ddd, 1H, J=11, 11 Hz), 2.75 (ddd, 1H, J=3.6, 10, 10 Hz), 3.21 (dd, 1H, J=7, 11.3 Hz), 3.80 (m, 1H), 3.89 (s, 3H, OMe), 4.16 (dd, 1H, J=1.5, 9.4 Hz), 4.26 (dd, 1H, J=5.2, 10.7 Hz), 4.46 (dd, 1H, 4, 10.6 Hz), 5.10 (s, 1H), 6.96 (d, 2H, J=8.8 Hz), 7.17 - 7.41 (m, 12H).

(Step 2) Preparation of (3S,4S,5R,6R)-N-banzhydryl-3-tertbutyldimethylsilyloxy-4-hydroxy-5,6-O-[(4-methoxy phenyl)ethylidene]piperidine

L-Selectride (23.2 mL, 23.2 mmol) was added to the title compound of the step 1 (3.3 g, 5.8 mmol) which was dissolved in tetrahydrofuran under nitrogen

and stirred for 17 hours. Distilled water and methylene chloride were added to the reaction mixture, the organic layer was separated, dried by sodium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 5~10 % ethylacetate/hexane solvent system to give the desired compound (2.2 g, 66 %).

^{1}H NMR ($CDCl_3$) • -0.36 (s, 3H), 0.03 (s, 3H), 0.84 (s, 9H), 1.58 (s, 3H), 2.29 (dd, 1H, J=10.5, 10.5 Hz), 2.54 (dd, 1H, J=4.8, 11 Hz), 2.89 (ddd, 1H, J=4.2, 10.5, 10.5 Hz), 3.55 (dd, 1H, J=2.5, 9 Hz), 3.61 (dd, 1H, J=10.5, 10.5 Hz), 3.74 (ddd, 1H, J=2.9, 4.7, 10.4 Hz), 3.87 (s, 1H), 4.45 (dd, 1H, J=4.3, 10.4 Hz), 4.95 (s, 1H), 6.97 (d, 2H, J=8.7 Hz), 7,17•7.39 (m, 12H).

(Step 3) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-methoxy-5,6-O-[(4-methoxy phenyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 6 of the Example 1.

^{1}H NMR ($CDCl_3$) • -0.03 (s, 3H, Si-Me), 0.03 (s, 3H, Si-Me), 0.85 (s, 9H, Si-tBu), 1.58 (s, 3H, Me), 2.33 (dd, 1H, J=11, 11 Hz), 2.51 (dd, 1H, J=4.4, 11 Hz),

2.78 (ddd, 1H, J=4.2, 10.4, 10.4 Hz), 3.47 (dd, 1H, J=2.3 9.4 Hz), 3.57 (m, 2H), 3.59 (s, 3H, OMe), 3.69 (ddd, 1H, J=2.6, 4.5, 4.5 Hz), 3.87 (s, 3H, OMe), 4.41 (dd, 1H, J=4.2 10.5 Hz), 4.93 (s, 1H), 6.96 (d, 2H, J=8.8 Hz), 7.17 - 7.38 (m, 12H).

(Step 4) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-hydroxy-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene] piperidine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.53 (s, 3H, Me), 2.03 (dd, 1H, J=10.5, 10.5 Hz), 2.74 (ddd, 1H, J=5.4, 11, 11 Hz), 2.78 (m, 1H), 3.55 - 3.68 (m, 4H), 3.64 (s, 3H, OMe), 3.87 (s, 3H, OMe), 4.44 (dd, 1H, J=4.3, 10.4 Hz), 4.93 (s, 1H), 6.95 (d, 2H, J=8.8 Hz), 7.17 - 7.39 (m, 12H).

(Step 5) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-methanesulfoxy-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.57 (s, 3H, Me), 2.52 (dd, 1H,

J=10.8, 10.8 Hz), 2.75 (dd, 1H, J=5, 10.7 Hz), 2.84 (ddd, 1H, J=6, 9.6, 9.6 Hz), 2.97 (s, 3H, OMs), 3.57 (d, 1H, J=10.2 Hz), 3.56 (m, 1H), 3.57 (s, 3H, OMe), 3.88 (s, 3H, OMe), 3.94 (m, 1H), 4.44 (dd, 1H, J=4.3, 10.4 Hz), 4.66 (ddd, 1H, J=2.8, 4.6, 10.9 Hz), 4.97 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.16 - 7.40 (m, 12H).

(Step 6) Preparation of {(3R,4S,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 9 of the Example 1.

^1H NMR ($CDCl_3$) • 1.56 (s, 3H, Me), 2.72 (m, 2H), 3.03 (ddd, 1H, J=4.2, 10, 10 Hz), 3.42 (m, 1H), 3.45 (s, 3H, OMe), 3.69 (dd, 1H, J=10.6, 10.6 Hz), 3.76 (m, 1H), 3.86 (s, 3H, OMe), 4.52 (dd, 1H, J=4, 10.4 Hz), 4.90 (m, 1H), 5.04 (s, 1H), 6.98 (d, 2H, J=8.7 Hz), 7.15 - 7.43 (m, 12H), 7.94 (s, 1H), 8.37 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4methoxyphenyl)ethylidene] piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.56 (s, 3H, Me), 2.76 (m, 2H), 3.07 (ddd, 1H, J=4.2, 10, 10 Hz), 3.48 (s, 3H, OMe), 3.69 (dd, 1H, J=10.6, 10.6 Hz), 3.79 (m, 2H), 3.90 (s, 3H, OMe), 4.52 (m, 1H), 4.98 (m, 1H), 5.05 (s, 1H), 7.00 (d, 2H, J=8.6 Hz), 7.18 - 7.56 (m, 15H), 8.04 (d, 2H, J=8.5 Hz), 8.17 (s, 1H), 8.81 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) • 2.93 (d, 1H, J=9.6 Hz), 2.99 (dd, 1H, J=3.9, 10.9 Hz), 3.20 (s, 3H, OMe), 3.88 (dd, 1H, 3.4, 3.4 Hz), 4.16 (dd, 1H, J=10.7, 10.7 Hz), 4.26 (dd, 1H, J=3.8, 12.2 Hz), 5.08 (dd, 1H, J=4, 9.5 Hz), 5.48 (s, 1H), 7.18 - 7.40 (m, 10H), 7.53 (t, 2H, J=7 Hz), 7.61 (d, 1H, J=7.1 Hz), 8.06 (d, 2H, J=7.3 Hz), 8.03 (s, 1H), 8.80 (s, 1H)).

(Step 9) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title

compound of the step 8 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) • 2.89 (m, 1H), 3.01 (m, 1H), 3.18 (m, 2H), 3,23 (s, 3H, OMe), 3.58 - 3.66 (m 2H), 3.82 (s, 6H, OMe), 4.36 (m 1H), 4.85 (m, 1H), 5.14 (m, 1H), 6.85 (d, 2H, J=8.9 Hz), 6.86 (d, 2H, J=8.8 Hz), 7.11 - 7.47 (m, 20H), 7.54 (t, 2H, J=7.5 Hz), 7.61 (d, 1H, J=7.3 Hz), 8.07 (d, 3H, J=7.9 Hz), 8.80 (s, 1H).

(Step 10) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 9 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) • 0.99 (d, 6H, J=6.6 Hz), 1.22 (d, 6H, J=6.6 Hz), 1.28 (d, 6H, J=6.8 Hz), 1.29 (d, 6H, J=6.8 Hz), 2.57 (m, 2H), 2.77 (dd, 2H, J=6.4, 6.8 Hz), 2.84 (m, 1H), 3.00 (m, 1H), 3.36 (s, 3H, OMe), 3.44 - 3.66 (m, 4H), 3.75 (m, 1H), 3.83 (s, 6H, OMe), 3.84 (s, 6H, OM e), 4.13 - 4.17 (m, 2H), 4.45 (m, 1H), 4.55 (d, 1H, J=10 Hz), 5.16 (m, 1H), 6.87 (d, 4H, J=7.4 Hz), 7.13 - 7.43 (m, 21H), 7.55 (t, 2H, J=7 Hz), 7.62 (d, 1H, J=7 Hz), 8.10 (d, 2H, J=7.3 Hz), 8.73 (s, 1H), 9.18 (s,

1H).

^{31}P NMR ($CDCl_3$) •: 153.08

Example 6 : Preparation of 6-N-benzoyl-{(3S,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine

(Step 1) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-ethoxy-5,6-O-[(4-methoxyphe-nyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 2 in Example 5 via the procedure described in the step 6 of the Example 1 using iodoethane (EtI).

^1H NMR ($CDCl_3$) • -0.05 (s, 3H, Si-Me), 0.01 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.18 (t, 3H, J=7 Hz, OCH_2CH_3), 1.51 (s, 3H, Me), 2.34 (dd, 1H, J=11.2, 11.2 Hz), 2.50 (dd, 1H, J=5, 10 Hz), 2.84 (ddd, 1H, J=4, 10, 10 Hz), 3.46 (dd, 1H, J=9.3 Hz), 3.56 (dd, 1H, J=10.4, 10.4 Hz), 3.67 (m 2H), 3.81 (q, 2H, J=7 Hz), 3.87 (s, 3H, OMe), 4.40 (dd, 1H, J=4, 10.4 Hz), 4.91 (s, 1H), 6.98 (d, 2H, J=8.7 Hz), 7.17 - 7.38 (m, 12H).

(Step 2) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-hydroxy-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]

piperidine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.18 (t, 3H, J=7.2 Hz, OCH_2CH_3), 1.52 (s, 3H, Me), 2.04 (dd, 1H, J=11, 11 Hz), 2.72 (dd, 1H, J=5, 11 Hz), 2.80 (m, 1H), 3.53•3.77 (m, 3H), 3.77 (dd, 1H, J=3, 3 Hz), 3.87 (s, 3H), 4.10 (m, 2H), 4.43 (dd, 1H, J=4, 10 Hz), 4.92 (s, 1H), 6.96 (d, 2H, J=8.8 Hz), 7.17•7.39 (m, 12H)

(Step 3) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-methanesulfonyl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethyl-idene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.17 (t, 3H, J=7 Hz, OCH_2CH_3), 1.62 (s, 3H, Me), 2.56 (dd, 1H, J=11, 11 Hz), 2.76 (dd, 1H, J=5, 10 Hz), 2.91 (m, 1H), 2.95 (s, 3H, OMs), 3.58 (m, 1H), 3.59 (dd, 1H, J=11, 11 Hz), 3.74 (dq, 1H, J=3, 7Hz, OCH_2CH_3), 3.87 (s, 3H, OMe), 3.93 (dq, 1H, J=3, 7 Hz, OCH_2CH_3), 4.03 (m, 1H), 4.39 (m, 1H), 4.65 (m, 1H), 4.96 (s, 1H), 6.97 (d, 2H, J=12.7 Hz), 7.18•7.41 (m, 12H).

(Step 4) Preparation of (3R,4S,5R,6R)-N-benzhydryl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-iladenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.02 (t, 3H, J=7 Hz, OCH_2CH_3), 1.53 (s, 3H, Me), 2.73 (m, 1H), 2.77 (d, 1H, J=12.4 Hz), 3.06 (ddd, 1H, J=4, 9, 9 Hz), 3.33 (dq, 1H, J=7, 11.8 Hz, OCH_2CH_3), 3.68 (dd, 1H, J=11, 11 Hz), 3.84•3.93 (m, 3H), 3.86 (s, 3H, OMe), 4.49 (dd, 1H, J=4.2, 10.5 Hz), 4.89 (ddd, 1H, J=2.4, 5, 11.3 Hz), 5.03 (s, 1H), 6.99 (d, 2H, J=8.7 Hz), 7.15•7.43 (m, 12H), 7.96 (s, 1H), 8.38 (s, 1H).

(Step 5) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene] piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) • 1.04 (t, 3H, J=7 Hz, OCH_2CH_3), 1.55 (s, 3H, Me), 2.77 (m, 2H), 3.04 (m, 1H), 3.39 (dd, 1H, J=7, 10 Hz), 3.70 (dd, 1H, J=10.5, 10.5 Hz), 3.87

(m, 2H), 3.90 (s, 3H, OMe), 3.96 (dd, 1H, J=7.2, 9.5 Hz), 4.50 (dd, 1H, J=4.2, 10.5 Hz), 4.99 (m, 1H), 5.05 (s, 1H), 6.99 (d, 2H, J=8.7 Hz), 7.17•7.56 (m, 15H), 8.03 (d, 2H, J=7 Hz), 8.19 (s, 1H), 8.82 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-ethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) • 0.96 (t, 3H, J=7 Hz, OCH_2CH_3), 2.91 - 3.18 (m, 3H), 3.19 (dq, 1H, J=7, 7 Hz, OCH_2CH_3), 3.38 (dq, 1H, J=7, 9.3 Hz, OCH_2CH_3), 3.98 (m, 1H), 4.14 (d, 1H, J=11.7 Hz), 4.16 (m, 1H), 4.25 (dd, 1H, J=3.7, 11.9 Hz), 5.08 (m, 1H), 5.48 (s, 1H), 7.8 - 7.63 (m, 13H), 8.06 (d, 2H, J=7 Hz), 8.40 (s, 1H), 8.80 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) • 0.95 (t, 3H, J=7 Hz, OCH_2CH_3),

2.91 (dd, 1H, J=7, 11.7 Hz), 3.04 (m, 1H), 3.15 (m, 1H), 3.25 (m, 1H), 3.38 (m, 1H), 3.56 (m, 1H), 3.62 (m, 1H), 3.82 (s, 6H, OMe), 3.95 (dd, 1H, J=4, 4 Hz), 4.33 (m, 1H), 4.83 (s, 1H), 5.12 (m, 1H), 6.84 (d, 2H, J=9 Hz), 6.87 (d, 2H, J=9 Hz), 7.08 - 7.58 (m, 18H), 7.47 (d, 2H, J=7 Hz), 7.55 (t, 2H, J=7 Hz), 7.62 (d, 1H, J=7.2 Hz), 8.06 (d, 2H, J=7.4 Hz), 8.81 (s, 1H), 9.06 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) • 0.88 (t, 3H, J=7 Hz, OCH_2CH_3) 0.96 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 1.20 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 1.22•1.30 (m, 6H, OMe), 1.97 (m, 1H), 2.29 (m, 1H), 2.53 (t, 1H), 2.84 (m, 1H), 2.90 (m, 1H), 3.02 (m, 1H), 3.50•3.59 (m, 7H), 8.84 (s, 6H), 4.08 (m, 1H), 4.38 (m, 1H), 4.51 (m, 1H), 5.13 (m, 1H), 6.87 (d, 4H, J=7 Hz), 7.03•7.40 (m, 19H), 7.56 (t, 2H, J=7.6 Hz), 7.62 (d, 1H, J=7.3 Hz), 8.10 (d, 2H, J=7 Hz), 8.81 (s, 1H), 9.14 (s, 1H)

^{31}P NMR ($CDCl_3$) •: 153.32

Example 7 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-6-hydroxymethyl-5-hydroxy-4-methoxypiperidine-3-yl} adenine

15 ml of ethylenechloride and 15 ml of trifluoroacetic acid were added to the compound (508 mg, 0.76 mmol) prepared from the step 10 of Example 2, and the resulting mixture was stirred at room temperature for 4 hours. After the reaction mixture was solidified under reduced pressure, this solid was purified by column chromatography eluted with 10%-25% methanol/methylene chloride to give 268 mg of the desired compound (95% yield).

^{1}H NMR (CD_3OD) δ 3.03 (s, 3H), 3.32 (m, 1H), 3.61 (dd, 1H, J=4.9, 12 Hz), 3.75 (dd, 1H, J=9.3, 9.3 Hz), 3.77 (dd, 1H, J= 12, 12 Hz), 3.88 (m, 2H), 4.07 (dd, 1H, J=9, 9 Hz), 4.75 (m, 1H), 7.50 (t, 1H, J=7.3 Hz), 7.61 (d, 1H, J=7 Hz), 8.48 (s, 1H), 8.68 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-6-hydroxymethyl-5-hydroxy-4-methoxypiperidine-3-yl}adenine

Methyliodide (42 μL, 0.7 mmol), 4-dimethylamino pyridine (catalytic amount) and triethylamine (273 μL, 1.75 mmol) were added to the title compound (140 mg, 0.35 mmol) prepared from step 1. The reaction mixture was stirred at room temperature for 15 hours, and concentrated under reduced pressure. The residue was dissolved in methylene chloride, the insoluble precipitate was removed by filtering, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography eluted with 10% - 25% methanol/methylene chloride to give 69 mg of the desired compound (48% yield).

^{1}H NMR (CD_3OD) δ 2.13 (m, 1H), 2.47 (s, 3H, N-Me), 3.10 (dd, 1H, J=5, 11 Hz), 3.17 (s, 3H, OMe), 3.22 (m, 1H), 3.72 (dd, 1H, J=9.5, 9.5 Hz), 3.95 (m, 2H), 3.99 (dd, 1H, J=9, 9 Hz), 4.67 (ddd, 1H, J=4.5, 11, 11 Hz), 7.59 (t, 2H, J=7.1 Hz), 7.67 (d, 1H, J=7.3 Hz), 8.11 (d, 2H, J=7.2 Hz), 8.54 (s, 1H), 8.74 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H, N-Me), 2.36 (m, 1H), 2.96 (d, 1H, J=2.5 Hz), 3.02 (dd, 1H, J=4.4, 11.2 Hz), 3.11 (s, 3H, OMe), 3.22 (dd, 1H, J=11.4, 11.5 Hz), 3.45 (dd, 1H, J=4.1, 10.1 Hz), 3.55 (dd, 1H, J=2.3. 8.7 Hz), 3.80 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.97 (dd, 1H, J=8.6, 10.3 Hz), 4.56 (ddd, 1H, J=4.7, 11.3, 11.3 Hz), 6.88 (d, 4H, J=8.7 Hz), 7.17 – 7.65 (m, 12H), 8.00 (s, 1H), 8.05 (d, 2H, J=7.4 Hz), 8.84 (s, 1H).

(Step 4) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.04 (d, 3H, J=6.8 Hz), 1.08 (d, 3H, J=6.8 Hz), 2.32 (t, 2H, J=6.8 Hz), 2.76 (m, 1H), 2.91 (m, 1H), 3.00 (s, 3H, N-Me), 3.04 (m, 1H), 3.15 (dd, 1H, J=7.3, 10.1 Hz), 3.30 – 3.70 (m, 5H), 3.81 (s, 6H, OMe), 3.83 (m, 1H), 4.23 (dd, 1H, J=3.8, 5.7 Hz), 4.55 (ddd, 1H, J=4, 9.2, 9.2 Hz), 6.84 (d, 2H, J=8.8 Hz), 6.87 (d, 2H, J=8.8 Hz), 7.21 – 7.62 (m, 12H), 8.05 (d, 2H, J=7.3 Hz), 8.18 (s, 1H), 8.86 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 151.14, 151.84 (major)

Example 8 : Preparation of 6-N-benzoyl-{(3R, 4R, 5R, 6R)-N-propyl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl}adenine

Under nitrogen atmosphere, the title compound prepared from the step 1 of Example 7 (240 mg, 0.6 mmol) was dissolved in 10 mL of anhydrous acetonitrile, to this resulting solution were added 4-dimethylaminopyridine (small amount), triethylamine (0.83 mL, 6 mmol) and n-propyliodide (300 μL, 3 mmol). This reaction mixture was heated to reflux for 4 hours, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 7 - 25% methanol/dichloromethane (CH_2Cl_2:MeOH=10:1) in order to give triethylamine salt and 200 mg of the desired compound (45% yield).

^1H NMR ($CDCl_3$) δ 0.90 (t, 3H, J=7.2 Hz, N-$CH_2CH_2C\underline{H}_3$), 1.52 (m, 2H, N-$CH_2C\underline{H}_2CH_3$), 2.54 (m, 2H), 2.75 (m, 1H), 3.05 (s, 3H, OMe), 3.17 (m, 1H), 3.33 (dd, 1H, J=11.4, 11.5 Hz), 3.83 (dd, 1H, J=9, 9 Hz), 3.90 - 4.10 (m, 3H), 4.50 (ddd, 1H, J=4.3, 10.6, 10.6 Hz), 7.54 (t,

2H, J=7.6 Hz), 7.62 (d, 1H, J=6.9 Hz), 8.05 (d, 2H, J=7.6 Hz), 8.05 (d, 2H, J=7.6 Hz), 8.06 (s, 1H), 8.82 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.67 (t, 3H, J=7.3 Hz, N-$CH_2CH_2C\underline{H}_3$), 1.32 (m, 2H, N-$CH_2C\underline{H}_2CH_3$), 2.30 (m, 1H), 2.48 (m, 1H), 2.64 (m, 1H), 3.05 (m, 1H), 3.10 (s, 3H, OMe), 3.23 (dd, 1H, J=11.3, 11.3 Hz), 3.45 (dd, 1H, 3.7, 10.1 Hz), 3.53 (dd, 1H, J=3.3, 10.1 Hz), 3.80 (s, 6H, OMe), 3.82 (m, 1H), 3.93 (dd, 1H, J=8.6, 10 Hz), 4.52 (ddd, 1H, J=4.3, 10.7, 10.7 Hz), 6.86 (d, 4H, J=8.7 Hz), 7.21 - 7.64 (m, 15H), 8.03 (s, 1H), 8.04 (d, 2H, J=8.5 Hz), 8.82 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from using title compound of the step 2 via the procedure described in

the step 13 of the Example 1.

^1H NMR ($CDCl_3$) δ 0.85 (t, 3H, J=7 Hz, N-$CH_2CH_2C\underline{H}_3$), 0.88 (t, 3H, J= 7 Hz, N-$CH_2CH_2C\underline{H}_3$), 0.94 (d, 6H, J=6.7 Hz, NCH($C\underline{H}_3$)$_2$)), 1.07 (d, 6H, J=6.7 Hz, NCH($C\underline{H}_3$)$_2$), 1.14 (d, 6H, J=6.8 Hz, NCH($C\underline{H}_3$)$_2$), 1.18 (d, 6H, J=6.8 Hz, NCH($C\underline{H}_3$)$_2$), 2.33 (t, 4H, J=6.3 Hz), 2.4 - 2.57 (m, 4H), 2.75 (t, 2H, J=6 Hz), 2.82 (m, 1H), 2.87 (m, 1H), 3.08 (m, 1H), 3.13 (m, 1H), 3.22 (s, 3H, OMe), 3.26 – 3.69 (m, 14H), 3.31(s, 3H, OMe), 3.81 (s, 6H, OMe), 4.23 (m, 3H), 4.29 (m, 1H), 4.33 (m, 1H), 4.63 (m, 1H), 4.74 (m, 2H), 6.84 (d, 4H, J=8.8 Hz), 6.85 (d, 4H, J=8.8 Hz), 7.21 – 7.64 (m, 24H), 8.05 (d, 2H, J=8.6 Hz), 8.64 (s, 1H), 8.80 (s, 1H), 8.81 (s, 1H), 8.92 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 149.94, 150.64

Example 9 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-6-hydroxymethyl-5-hydroxy-4-methoxypiperidine-3-yl}adenine

Under nitrogen atmosphere, the title compound prepared from the step 1 of Example 7 (300 mg, 0.78 mmol) was dissolved in 10 mL of anhydrous acetonitrile,

and to the resulting solution were added 4-dimethylaminopyridine (small amount), triethylamine (1.08 mL, 7,8 mmol) and benzylbromide (468 μL, 3.9 mmol). This reaction mixture was heated to reflux for 4 hours, and solvent was evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 7-25% methanol/dichloro-methane (CH_2Cl_2 : MeOH = 15:1-4:1) to give 66 mg of the desired compound (18% yield).

^{1}H NMR ($CDCl_3$) δ 2.84 - 2.97 (m, 2H), 3.19 (dd, 1H, J=11.2, 11.2 Hz), 3.36 (m, 1H), 3.51 (s, 3H, OMe), 3.73 (d, 1H, J=9 Hz), 3.85 (ddd, 1H, 5, 9.2, 9.2 Hz), 3.99 (dd, 1H, J=9, 9 Hz), 4.09 (m, 2H), 4.32 (dd, 1H, J=6.7, 6.7 Hz), 7.23 - 7.45(m, 8H), 7.94(d, 2H, J=7.1 Hz), 8.04 (s, 1H), 8.41 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.70 (m, 1H), 2.98 (dd, 1H, J=4.6, 11.6 Hz), 3.07 (s, 3H, OMe), 3.15 (m, 2H), 3.61 (m, 2H), 3.803 (s, 3H, OMe), 3.807 (s, 3H, OMe), 3.89 - 4.02 (m,

3H), 4.44 (ddd, 1H, J=4, 10, 10 Hz), 6.84 (d, 2H, J=8.93 Hz), 6.85 (d, 2H, J=8.9 Hz), 7.18 – 7.56 (m, 16H), 7.61 (d, 2H, J=7.2 Hz), 7.96(s, 1H), 8.03 (d, 2H, J=7.2 Hz), 8.81 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-[(cyanoethoxy) (N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.96 (d, 3H, J=6.7 Hz), 1.08 (d, 3H, J=6.7 Hz), 1.12 (d, 3H, J=7 Hz), 1.14 (d, 3H, J=7 Hz), 2.37 (t, 1H, J=6.3 Hz), 2.43 (t, 1H, J=6.4 Hz), 2.80 (m, 1H), 3.34 (s, 2H, OMe), 3.10 – 3.79 (m, 10H), 3.81 (s, 3H, OMe), 3.82 (s, 3H, OMe), 4.32 (m, 1H), 4.58 (m, 1H), 4.73 (m, 1H), 6.84 (d, 4H, J=8.9 Hz), 7.21 – 7.64 (m, 18H), 8.04 (d, 2H, J=7.4 Hz), 8.42 (s, 1H), 8.82 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 150.53

Example 10 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-6-hydroxymethyl-5-hydroxy-4-methoxy piperidine-3-yl}adenine

The title compound of the step 1 of Example 7 (450 mg, 1.17 mmol) was dissolved in 40 mL of anhydrous acetonitrile, and to the resulting solution were added α-bromo-p-tolunitrile (1 g, 5.85 mmol), triethylamine (1.62 mL, 10.7 mmol) and dimethylaminopyridine (small amount). This reaction mixture was stirred at room temperature for 14 hours, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography eluted with 5-25% methanol/methylene chloride to give 200 mg of the desired compound (32% yield).

^{1}H NMR ($CDCl_3$) δ 2.63 (m, 1H), 2.92 (dd, 1H, J=4.2, 11.3 Hz), 3.07 (s, 3H, OMe), 3.23 (m, 1H), 3.43 (d, 1H, J=14.2), 3.90 (dd, 1H, J=9, 9 Hz), 3.97 - 4.16 (m, 4H), 4.33 (d, 1H, J=14.3 Hz), 4.44 (ddd, 1H, J=4, 10.6, 10.6 Hz), 7.46 (m, 2H), 7.52 (d, 2H, J=7.7 Hz), 7.59 (d, 3H, J=8.3 Hz), 8.01 (d, 2H, J=9.8 Hz), 8.03 (s, 1H), 8.76 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.75 (m, 1H), 2.86 (dd, 1H, J=4.5, 11.2 Hz), 3.07 (s, 3H, OMe), 3.20 (dd, 1H, J=5.4, 11 Hz), 3.30 (dd, 1H, J=5.7, 8 Hz), 3.57 (m, 2H), 3.788 (s, 3H, OMe), 3.794 (s, 3H, OMe), 3.88 (dd, 1H, J=9, 9Hz), 3.97 (m, 2H), 4.39 (ddd, 1H, J=4, 10, 10 Hz), 6.80 (d, 2H, J=8.7 Hz), 6.81 (d, 2H, J=8.7 Hz), 7.25 - 7.54 (m, 16H), 7.97(s, 1H), 8.03 (d, 2H, J=7.2 Hz), 8.80 (s, 1H).

(Step 3)Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.05 (d, 6H, J=6.7 Hz), 1.09 (d, 6H, J=6.2 Hz), 1.11 (d, 6H, J=5.6 Hz), 1.15 (d, 6H, J=6.7 Hz), 2.02 - 2.06 (m, 1H), 2.21 - 2.40 (m, 2H), 2.44 (t, 2H, J=6.1 Hz), 2.95 (m, 2H), 3.07 (s, 3H, OMe), 3.31 (s, 3H, OMe), 3.31 - 3.82 (m, 13H), 3.80 (s, 6H, OMe), 3.88 - 3.99 (m, 4H), 4.13 (m, 2H), 4.50 (m, 2H),

4.73 (m, 2H), 5.38 (m, 2H), 6.82 (d, 4H; J=8.6 Hz), 6.83 (d, 4H, J=8.1 Hz), 7.16 - 7.46 (m, 23H), 7.54 (t, 4H, J=8 Hz), 7.61 (d, 2H, J=8.9 Hz), 8.04 (m, 5H), 8.19 (s, 1H), 8.65 (s, 1H), 8.81 (s, 1H), 8.84 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 150.69, 151.45

Example 11 : Preparation of 6-N-benzoyl-{(3R,$R,5R,6R)-N-(4-fluorobenzyl)-5-[(2-cyanoethoxy) (N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-6-hydroxymethyl-5-hydroxy-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound prepared from the step 1 of the Example and 4-fluorobenzylbromide via the procedure described in the step 1 of the Example 10.

^{1}H NMR ($CDCl_3$) δ 2.53 (m, 1H), 2.94 (dd, 1H, J=4.6 Hz), 3.2 (m, 2H), 3.94 (m, 2H), 4.02 (m, 2H), 4.15 (m, 2H), 4.42 (ddd, 1H, J=4.6, 10.4, 10.4 Hz), 7.46 (t, 2H, J=7.1 Hz), 7.52 (d, 2H, J=9.2 Hz), 7.64 - 7.54 (m, 3H), 7.97 (d, 2H, J=7.4 Hz), 8.01 (s, 1H), 8.72 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-5-hydroxy-6-dimethyltrityloxymethyl-4-

methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.69 (m, 1H), 2.93 (dd, 1H, J=4.5, 11.4 Hz), 3.06 (s, 3H, OMe), 3.14 (m, 1H), 3.35 - 3.28 (m, 2H), 3.59 (m, 2H), 3.80(s, 6H, OMe), 3.98 - 3.87 (m, 2H), 4.42 (ddd, 1H, J=4.6, 11.1, 11.1 Hz), 6.83 (d, 2H, J=6.5 Hz), 6.84 (d, 2H, J=7.1 Hz), 7.39 - 7.17 (m, 14H), 7.51 (t, 1H, J=7.0 Hz), 7.61 (d, 1H, J=7.5 Hz), 7.96 (s, 1H), 8.03 (d, 2H, J=7.2 Hz), 8.81 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.99 (d, 6H, J=6.8 Hz), 1.06 (d, 6H, J=6.0 Hz), 1.09 (d, 6H, J=6.1 Hz), 1.14 (d, 6H, J=6.8 Hz), 2.40 (m, 4H), 2.78 (m, 2H), 2.92 (m, 2H), 3.15 (s, 3H, OMe), 3.35 (s, 3H, OMe), 3.79 - 3.21 (m, 16H), 3.81 (s, 6H), 3.82 (s, 6H, OMe), 4.19 - 4.05 (m, 4H), 4.30 (m, 2H), 4.55 (m, 2H), 4.72 (m, 2H), 6.85 (d,

8H, J=8.8 Hz), 7.63 - 6.96 (m, 35H), 8.04 (d, 4H, J=8.2 Hz), 8.34 (s, 1H), 8.74 (s, 1H), 8.80 (s, 1H), 8.83 (s, 1H).

Example 12 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-[(2-cyanoethoxy) (N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-6-hydroxymethyl-5-hydroxy-4-methoxy piperidine-3-yl}adenine

The title compound prepared from the step 1 of Example 7 (200 mg, 0.5 mmol) was dissolved in 5 mL of anhydrous acetonitrile, to the resulting solution were added 4-methoxybenzylchloride (339 mg, 2.5 mmol), triethylamine (0.69 mL, 5 mmol) and dimethylamimo pyridine (small amount). This reaction mixture was heated to reflux for 2 hours, and concentrated under reduced pressure. The residue was purified by column chromatography eluted with 10-20% methanol/dichloromethane to give 104 mg of the desired compound (40 % yield).

^{1}H NMR ($CDCl_3$) δ 2.60 (d, 1H, J=9 Hz), 3.03 (s, 3H, OMe), 3.07 (m, 1H), 3.19 (dd, 1H, J=11.5, 11.5 Hz), 3.35 (d, 1H, J=13.4 Hz), 3.79 (s, 3H, OMe), 3.88 (dd,

1H, J=9, 9 Hz), 3.99 (dd, 1H, J=9, 9 Hz), 4.12 (m, 3H), 4.42 (ddd, 1H, J=4, 10.5, 10.5 Hz), 6.86 (d, 2H, J=8.6 Hz), 7.20 (d, 2H, J=8.6 Hz), 7.54 (t, 2H, J=7 Hz), 7.62 (d, 1H, J=7 Hz), 7.96 (s, 1H), 8.04 (d, 2H, J=7 Hz), 8.81 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.67 (m, 1H), 2.79 (m, 1H), 2.99 (dd, 1H, J=3, 9 Hz), 3.04 (m, 1H), 3.07 (s, 3H, OMe), 3.12 (m, 1H), 3.61 (m, 2H), 3.77 (s, 3H, OMe), 3.81 (s, 6H, OMe), 3.89 (d, 1H, J=9 Hz), 3.94 (m, 1H), 4.43 (ddd, 1H, J=4.7, 10, 10), 6.78 (d, 2H, J=8.7 Hz), 6.86 (d, 2H, J=8.6 Hz), 6.87 (d, 2H, J=8.7 Hz), 7.08 (d, 2H, J=8.5 Hz), 7.22□7.62 (m, 22H), 7.95 (s, 1H), 8.04 (d, 2H, J=7.4 Hz), 8.81 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title

compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.96 (d, 6H, J=6.7 Hz), 1.08 (d, 6H, J=6.7 Hz), 1.13 (d, 6H, J=6.7 Hz), 1.15 (d, 6H, J=6.7 Hz), 2.36 (t, 2H, J=6 Hz), 2.44 (t, 2H, J=6 Hz), 2.87 (dd, 2H, J= 4, 13 Hz), 3.10 (m, 4H), 3.19 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.77□3.36 (m, 12H), 3.79 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.82 (s, 6H, OMe), 4.33 (m, 1H), 4.58 (m, 1H), 4.72 (m, 1H), 6.85 (d, 4H, J=8.6 Hz), 6.88 (d, 4H, J=8.8 Hz), 7.50 - 7.11 (m, 13H), 7.53 (t, 2H, J=7.8 Hz), 7.61 (d, 1H, J=7.1 Hz), 8.05 (d, 2H, J=7.5 Hz), 8.45 (s, 2H), 8.77 (s, 2H).

^{31}P NMR ($CDCl_3$) δ: 150.41, 150.60

Example 13 : Preparation of 4-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}cytosine

(Step 1) Preparation of 4-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}cytosine

The title compound prepared from the step 7 of Example 1 (600 mg, 1.26 mmol) was dissolved in 30 mL of anhydrous dioxane, to this resulting solution were added the mixture of N^3-benzoylcytosine (600 mg, 1.26

mmol) and triphenylphosphine (1.5 g, 5.76 mmol). Diethylazodicarboxylate (1 mL, 1 mmol) dissolved in 12 mL of anhydrous tetrahydrofuran was added to this reaction mixture and stirred at room temperature overnight. Ethylacetate was added to the reaction mixture, and the resulting reaction mixture washed twice with 50 mL of distilled water. The organic layer was separated, water removed with sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 50-75% ethylacetate/hexane (30 - 50% EtOAc/Hexane) to give 200 mg of the desired compound (24% yield).

^1H NMR ($CDCl_3$) δ 1.55 (s, 3H, Me), 2.64 (m, 1H), 3.00 (dd, 1H, J=4.0, 11.2 Hz), 3.59 (s, 3H, OMe), 3.70 (m, 2H), 3.82 (m, 2H), 3.88 (s, 3H, OMe), 4.23 (m, 1H), 4.46 (dd, 1H, J=3.8, 10.5 Hz), 5.01 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.69 - 7.12 (m, 17H), 7.92 (d, 2H, J=7.6 Hz).

(Step 2) Preparation of 4-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl-6-hydroxymethyl-5-hydroxy-4- methoxy piperidine-3-yl}cytosine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.03 (m, 1H), 2.49 (m, 1H), 2.72 (m, 1H), 3.22 (dd, 1H, J=4,2, 12.0 Hz), 3.44 (s, 3H, OMe), 3.63 (m, 1H), 4.07 (m, 2H), 4.23 (dd, 1H, J=3.7, 12.3 Hz), 5.46 (s, 1H), 7.93 - 7.24 (m, 19H).

(Step 3) 4-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl) -5-hydroxy-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}cytosine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.52 (m, 1H), 2.58 (m, 1H), 3.09 (dd, 1H, J=3.8, 11.5 Hz), 3.41 (s, 3H, OMe), 3.62 (m, 1H), 3.77 (m, 2H), 3.79 (s, 3H, OMe), 3.80 (s, 3H, OMe), 4.17 (m, 2H), 5.04 (s, 1H), 6.80 (d, 2H, J=8.7 Hz), 6.81 (d, 2H, J=8.8 Hz), 7.72 - 7.13 (m, 24H), 7.90 (d, 2H, J=7.4 Hz).

(Step 4) 4-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} cytosine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 13 of the Example 1.

^1H NMR ($CDCl_3$) δ 1.17 (d, 3H, J=6.8 Hz), 1.21 (d, 3H, J=6.7 Hz), 2.64 - 2.42 (m, 4H), 2.91 (dd, 1H, J=4.8, 13.3 Hz), 3.09 (m, 1H, OMe), 3.35 (s, 3H OMe), 3.64 - 3.48 (m, 5H), 3.829 (s, 3H OMe), 3.832 (s, 3H OMe), 4.19 (m, 1H), 4.48 (d, 1H, J=12.8 Hz), 4.64 (s, 1H), 4.68 (m, 1H), 5.04 (s, 1H), 6.82 (m, 4H), 7.71 - 7.14 (m, 2H), 7.91 (d, 2H, J=8.3 Hz).

^{31}P NMR ($CDCl_3$) δ: 149.72, 150.59

Example 14: Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}guanine

(Step 1) Preparation of 2-N-isobutyryl—6-O-[2-(p-nitrophenyl)ethyl]-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}guanine

The title compound prepared from the step 7 of Example 1 was dissolved in 70 mL of anhydrous xylene, to this resulting solution were added N^2-isobutyryl-O^6-[2-(p-nitrophenyl)ethyl]guanine (2.3 g, 6.2 mmol) and triphenylphosphine (1.74 g, 6.6 mmol), and the reaction mixture was stirred at room temperature for 1 hour. The diethylcarboxylate (DEAD, 1.04 mL, 6.6 mmol) dissolved in 10 ml of anhydrous xylene, was slowly

added to this reaction mixture for 20 min, and stirred at 120°C for 6 hours. After slow cooling of the reaction mixture to room temperature, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluted with the mixed solvent of ethylacetate and hexane (1:1) to give 560 mg of the desired compound (22% yield).

^1H NMR ($CDCl_3$) δ 1.28 (d, 3H, J=4.9 Hz), 1.29 (d, 3H, J=5.3 Hz), 1.30 (d, 3H, J=4.6 Hz), 1.31 (d, 3H, J=5.3 Hz), 1.55 (s, 3H, Me), 1.81 (s, 3H, Me), 2.58 (m, 2H), 2.72 (dd, 2H, J=11.2, 11.3 Hz), 3.00 (dd, 1H, J=4.5, 11.2 Hz), 3.08 (m, 1H), 3.34 (t, 2H, J=6.9 Hz), 3.35 (s, 3H, OMe), 3.38 (s, 3H, OMe), 3.70 (d, 2H, J=9.5 Hz), 3.80 (d, 2H, J=9 Hz), 3.82 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.06 - 4.39 (m, 6H), 4.53 (m, 2H), 4.80 (m, 2H), 5.02 (s, 1H), 5.14 (s, 1H), 6.90 (d, 2H, J=8.9 Hz), 6.99 (d, 2H, J=8.8 Hz), 7.72 - 7.15 (m, 30H), 8.18 (d, 4H, J=8.7 Hz).

(Step 2) Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}guanine

The title compound of the step 1 was dissolved in 12 mL of anhydrous pyridine, and to this solution was added 1,8-diazabicyclo[5.4.0]undec-7-N (DBU, 203 mL,

1.35 mmol). The mixture was stirred at room temperature for 10 hours, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluted with 5-10% methanol/methylene chloride to give 375 mg of the desired compound (83% yield).

^{1}H NMR ($CDCl_3$) δ 1.310 (d, 3H, J=4.1 Hz), 1.32 (d, 3H, J=4 Hz), 1.33 (d, 3H, J=4.7 Hz), 1.34 (d, 3H, J=4.1 Hz), 1.56 (s, 3H, Me), 1.80 (s, 3H, Me), 2.51 (dd, 1H, J=11.2, 11.3 Hz), 2.62 (m, 3H), 3.02 (dd, 1H, J=4.5, 11.2 Hz), 3.08 (m, 1H), 3.40 (s, 3H, OMe), 3.45 (s, 3H, OMe), 3.69 (d, 2H, J=9.5 Hz), 3.77 (d, 2H, J=9 Hz), 3.82 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.27 - 4.05 (m, 6H), 4.58 - 4.45 (m, 2H), 5.02 (s, 1H), 5.13 (s, 1H), 6.90 (d, 2H, J=8.7 Hz), 6.99 (d, 2H, J=8.9 Hz), 7.72 - 7.15 (m, 26H).

(Step 3) Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-6-hydroxymethyl-5-hydroxy-4-methoxy piperidine-3-yl}guanine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.25 (d, 3H, J=7 Hz), 1.28 (d, 3H, J=7 Hz), 2.59 (d, 1H, J=8.5 Hz), 2.71 (dd, 1H, J=10.5,

10.5 Hz), 2.87 (dq, 1H, J=7.7 Hz), 3.07 (m, 1H), 3.16 (s, 3H, OMe), 3.65 (dd, 1H, J=9, 9 Hz), 4.06 (dd, 1H, J=8.6, 8.6 Hz), 4.21 (m, 2H), 4.41 (m, 1H), 5.56 (s, 1H), 7.30 - 7.12 (m, 10H), 7.86 (s, 1H).

(Step 4) Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}guanine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.26 (d, 3H, J=7.2 Hz), 1.30 (d, 3H, J=6.8 Hz), 2.56 (d, 1H, J=8.7 Hz), 2.66 (m, 2H), 3.08 (dd, 1H, J=4.2, 11.4 Hz), 3.15 (s, 3H, OMe), 3.29 (dq, 1H, J=1.5, 5.7 Hz), 3.57 (dd, 1H, J=8.9, 8.9 Hz), 3.67 (m, 1H), 3.77 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.81 (m, 1H), 4.39 (ddd, 1H, J=4, 11.6, 11.6 Hz), 5.06 (s, 1H), 6.80 (d, 2H, J=8.9 Hz), 6.81 (d, 2H, J=8.8 Hz), 7.48 - 7.22 (m, 15H), 7.65 (s, 1H).

(Step 5) Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}guanine

This compound was prepared from the title compound of the step 4 via the procedure described in

the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.15 (d, 6H, J=6.7 Hz), 1.16 (d, 6H, J=7.3 Hz), 1.19 (d, 6H, J=6.5 Hz), 1.21 (d, 6H, J=6.7 Hz), 1.26 (d, 6H, J=6.8 Hz), 1.27 (d, 6H, J=6.8 Hz), 2.38 (t, 4H, J=7 Hz), 2.94 - 2.42 (m, 10H), 3.18 (s, 3H, OMe), 3.24 (s, 3H, OMe), 3.30 (dd, 1H, J=5.7, 7 Hz), 3.40 (m, 1H), 3.80 - 3.53 (m, 10H), 3.812 (s, 3H, OMe), 3.818 (s, 3H, OMe), 4.17 (m, 1H), 4.53 - 4.37 (m, 3H), 4.67 (s, 1H), 4.72 (s, 1H), 6.81 (d, 2H, J=8.8 Hz), 6.83 (d, 2H, J=8.9 Hz), 7.13 - 7.35 (m), 8.32 (s, 1H), 8.63 (s, 1H).

^{31}P NMR ($CDCl_3$) δ: 149.32, 150.08

Example 15 : Preparation of {(3R,4R,5R,6R)-N-benzhydryl -5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy] -6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} thymine

(Step 1) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}thymine

The title compound prepared from the step 7 of Example 1 (2.2 g, 4.63 mmol) was dissolved in 100 mL of anhydrous dioxane, to this resulting solution were added N^3-benzoylthymine (2.7 g, 20.73 mmol) and triphenylphosphine (3.3 g, 12.58 mmol), and stirred.

To this reaction mixture was added diethyl azodicarboxylate (DEAD, 2.1 mL, 11.87 mmol) dissolved in 15 mL of anhydrous tetrahydrofuran, and the resulting reaction mixture was stirred at room temperature for 12 hours. The reaction mixture was concentration under reduced pressure, the residue was dissolved in 90 mL of methanol, and saturated with ammonia gas for 30 min. The saturated residue was concentrated under reduced pressure with adding toluene, and purified by silica gel column chromatography eluted with 30-50% ethylacetate/hexane in order to give 2.45 g of the desired compound (95% yield).

^{1}H NMR ($CDCl_3$) δ 1.79 (s, 3H, Me), 1.89 (s, 3H, Me), 2.54 (ddd, 1H, J=4, 10, 10 Hz), 3.01 (dd, 1H, J=4.3, 11.1 Hz), 3.56 (s, 3H, OMe), 3.45 (s, 3H, OMe), 3.62 (m, 1H), 3.82 (s, 3H, OMe), 4.12 (dd, 1H, J=7.1, 7.2 Hz), 4.15 (m, 3H), 4.52 (dd, 1H, J=4.2, 10.7 Hz), 5.09 (s, 1H), 6.86 (s, 1H, vinyl H), 6.89 (d, 2H, J=8.9 Hz), 7.12 - 7.47 (m, 12H).

(Step 2) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-6-hydroxymethyl-5-hydroxy-4-methoxypiperidine-3-yl} thymine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 11 of the Example 1.

^1H NMR ($CDCl_3$) δ 3.35 (s, 3H, OMe), 1.89 (s, 3H, Me), 2.53 (dd, 1H, J=8.3, 12.5 Hz), 3.08 (dd, 1H, J=4, 11.4 Hz), 3.33 (m, 1H), 3.35 (s, 3H, OMe), 3.54 (s, 3H, OMe), 3.77 (dd, 1H, J=8.4, 8.4 Hz), 3.97 (dd, 1H, J=8.2, 8.3 Hz), 4.10 (d, 1H, J=10.5 Hz), 4.22 (m, 2H), 5.46 (s, 1H), 7.05 (s, 1H, vinyl H), 7.20 - 7.41 (m, 10H).

(Step 3) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 12 of the Example 1.

^1H NMR ($CDCl_3$) δ 1.91 (s, 3H, Me), 2.47 (d, 1H, J=9 Hz), 2.48 (m, 1H), 3.03 (dd, 1H, J=4, 10.5 Hz), 3.44 (s, 3H, OMe), 3.53 - 3.79 (m, 5H), 3.791 (s, 3H, OMe), 3.797 (s, 3H, OMe), 5.05 (s, 1H), 6.80 (d, 4H, J=8.8 Hz), 6.81 (d, 4H, J=8.8 Hz), 6.83 (s, 1H, vinyl H), 7.22 - 7.37 (m, 15H), 7.47 (d, 2H, J=6.8 Hz).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} thymine

This compound was prepared from the title

compound of the step 3 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.27 (d, 6H, J=6.7 Hz), 1.29 (d, 6H, J=6.8 Hz), 1.91 (s, 3H, Me), 2.49 (d, 1H, J=8.5 Hz), 2.57 (d, 1H, J=3.1 Hz), 2.77 (t, 2H, J=6.2 Hz), 3.03 (dd, 1H, J=4.2, 11.4 Hz), 3.44 (s, 3H, OMe), 3.46 - 3.73 (m, 6H), 3.76 (m, 1H), 3.790 (s, 3H, OMe), 3.796 (s, 3H, OMe), 4.13 (m, 2H), 5.05 (s, 1H), 6.80 (d, 2H, J=8.8 Hz), 6.81 (d, 2H, J=8.9 Hz), 7.00 (s, 1H, vinyl H), 7.20 - 7.37 (m, 15H), 7.46 (d, 2H, J=7 Hz).

Example 16 : Preparation of 3-N-methyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}thymine

(Step 1) Preparation of 3-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}thymine

The title compound prepared from the step 7 of Example 1 (1.4 g, 2.94 mmol) was dissolved in 65 mL of anhydrous dioxane, to this solution were added N^3-benzoylthymine (1.7 g, 13.1 mmol) and triphenylphosphine (2.1 g, 8 mmol), and this resulting solution was stirred. To this reaction mixture was added diethyl azodicarboxylate (DEAD, 1.34 mL, 1.8

mmol) dissolved in 10 mL of anhydrous tetrahydrofuran, and stirred at room temperature for 12 hours. The reaction mixture was concentrated under reduced pressure, the residue was purified by silica gel column chromatography eluted with 30% ethylacetate/hexane in order to give 1.9 g of the desired compound (94% yield).

^{1}H NMR ($CDCl_3$) δ 1.78 (s, 3H, Me), 1.94 (s, 3H, Me), 2.54 (m, 1H), 3.04 (dd, 1H, J=3.3, 10 Hz), 3.62 (s, 3H, OMe), 3.68 (m, 1H), 3.82 (s, 3H, OMe), 4.01 (dd, 1H, J=10, 10 Hz), 4.13 - 4.32 (m, 3H), 4.51 (dd, 1H, J=7, 10.5 Hz), 5.08 (s, 1H), 6.89 (s, 2H, J=8.8 Hz), 6.96 (s, 1H, vinyl H), 7.13 - 7.51 (m, 12H), 7.64 (d, 1H, J=7.4 Hz), 7.91 (d, 2H, J=7.2 Hz).

(Step 2) Preparation of 3-N-benzoyl-{(3R,4R,5R,6R)-6-hydroxymethyl-5-hydroxy-4- methoxypiperidine-3-yl} thymine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 1 of the Example 7.

^{1}H NMR (CD_3OD) δ 1.99 (s, 3H, Me), 3.19 (ddd, 1H, J=4.2, 4, 10.3 Hz), 3.32 (m, 2H), 3.58 (m, 1H), 3.60 (s, 3H, OMe), 3.74 (dd, 1H, J=10.4, 10.4 Hz), 3.89 (m, 1H), 3.91 (m, 2H), 7.50 (t, 1H, J=7.6 Hz), 7.74 (s, 1H), 7.75 (d, 1H, J=7.5 Hz), 7.96 (d, 2H, J=7.2 Hz).

[redacted]

(Step 3) Preparation of 3-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 2 of the Example 7.

^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H, Me), 2.02 (m, 1H), 2.36 (s, 3H, NMe), 2.80 (m, 1H), 2.99 (dd, 1H, J=4, 4, 10.8 Hz), 3.51 (s, 3H, OMe), 3.85 - 3.72 (m, 4H), 3.96 (d, 1H, J=11 Hz), 7.13 (s, 1H, vinyl H), 7.50 (t, 1H, J=7.5 Hz), 7.65 (d, 1H, J=7.4 Hz), 7.92 (d, 2H, J=7.2 Hz).

(Step 4) Preparation of [(3R,4R,5R,6R)-N-methyl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl} thymine

The title compound of the above step 3 (50 mg, 0.12 mmol) was dissolved in 5 mL of methanol, saturated at 0°C with ammonia gas for 10 min, and stirred at room temperature for 10 min. The oversaturated ammonia gas was removed through stirring of the open reaction system. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography eluted with 10 - 25% methanol/methylene chloride to give 26 mg of the

desired compound (73% yield).

^{1}H NMR (CD_3OD) δ 1.91 (s, 3H, Me), 1.95 (m, 1H), 2.40 (s, 3H, NMe), 2.60 (m, 1H), 2.88 (dd, 1H, J=4.4, 11 Hz), 3.44 (m, 1H), 3.46 (s, 3H, OMe), 3.61 (m, 2H), 3.89 (d, 2H, J=2.5 Hz), 7.59 (s, 1H, vinyl H).

(Step 5) Preparation of {(3R,4R,5R,6R)-N-methyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H, Me), 2.14 (s, 3H, NMe), 2.17 (m, 1H), 2.65 (m, 1H), 2.86 (dd, 1H, J=4.2, 11 Hz), 3.42 (m, 1H), 3.45 (s, 3H, OMe), 3.48 (m, 1H), 3.65 (m, 1H), 3.77 (m, 1H), 3.80 (s, 6H, OMe), 4.23 (dd, 1H, J=3.8, 5.8 Hz), 6.49 (d, 2H, J=5.1 Hz), 6.50 (d, 2H, J=5 Hz), 7.01 (s, 1H, vinyl H), 7.48 - 7.22 (m, 9H).

(Step 6) Preparation of {(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} thymine

This compound was prepared from the title compound of the step 5 via the procedure described in

the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.11 (d, 6H, J=6.5 Hz), 1.12 (d, 12H, J=6.7 Hz), 1.16 (d, 6H, J=6.8 Hz), 1.93 (s, 6H, Me), 2.30 (s, 3H, NMe), 2.34 (s, 3H, NMe), 2.55 (t, 4H, J=6 Hz), 2.79 - 2.63 (m, 3H), 2.86 (m, 3H), 3.11 (dd, 1H, J=7, 10 Hz), 3.23 (dd, 1H, J=5.6, 10 Hz), 3.33 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.74 - 3.38 (m, 15H), 3.81 (s, 12H, OMe), 4.15 (m, 2H), 4.20 (m, 2H), 4.38 (m, 1H), 6.86 - 6.82 (m, 8H), 7.47 - 7.28 (m, 20H).

^{31}P NMR ($CDCl_3$) δ: 151.73, 151.90

Example 17 : Preparation of {(3R,4R,5R,6R)-N-fluorenyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} thymine

(Step 1) Preparation of {(3R,4R,5R,6R)-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound prepared from the step 1 of the Example 15 via the procedure described in the step 1 of Example 7.

^{1}H NMR (DMSO) δ 1.80 (s, 3H, Me), 3.16 (m, 1H), 3.28 (m, 2H) 3.33 (s, 3H, OMe), 3.53 - 3.66 (m, 4H), 3.78 (m, 1H), 5.47 (s, 1H), 7.74 (s, 1H, vinyl H).

(Step 2) Preparation of {(3R,4R,5R,6R)-N-fluorenyl-5-hydroxy-6-dimethyltrityloxymethylhydroxymethyl-4-methoxypiperidine-3-yl}thymine

The title compound of the above step 1 (800 mg, 2.8 mmol) was dissolved in 18 mL of dioxane, and to this resulting mixture were added 9-fluorenylmethoxycarbonyl chloride (1.04 g, 2.32 mmol) and 35 mL of 10 % sodium carbonate solution at 0°C . After stirring of this reaction mixture at 0°C for 1 hour, this reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluted with 50% ethylacetate/hexane to give 600 mg of the desired compound (42% yield).

^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H, Me), 3.37 (m, 1H), 3.46 (s, 3H, OMe), 3.50 (m, 5H), 3.89 (m, 2H), 4.25 (dd, 1H, J=5.4, 5.4 Hz), 4.55 (dd, 1H, J=5.3, 10.5 Hz), 4.70 (dd, 1H, J=5.5, 10.5 Hz), 6.85 (s, 1H, vinyl H), 7.33 (t, 2H, J=7.3 Hz), 7.42 (t, 2H, J=7.3 Hz), 7.57 (dd, 2H, J=4, 7.3 Hz), 7.77 (d, 2H, J=7.5 Hz).

(Step 3) Preparation of {(3R,4R,5R,6R)-N-fluorenyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 2 via the procedure described in

the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.81 (s, 3H, Me), 3.40 (m, 2H), 3.41 (s, 3H, OMe), 3.58 (m, 1H), 3.761 (s, 3H, OMe), 3.763 (s, 3H, OMe), 3.94 - 4.17 (m, 4H), 4.26 (dd, 1H, J=6.3, 10.5 Hz), 4.63 (m, 2H), 6.89 (d, 4H, J=9 Hz), 7.03 (s, 1H, vinyl H), 7.22 - 7.77 (m, 15H), 8.24 (dd, 2H, J=1.5, 5 Hz).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-fluorenyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} thymine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.04 (d, 6H, J=6.5 Hz), 1.09 (d, 6H, J=6.7 Hz), 1.15 (d, 12H, J=6.3 Hz), 1.79 (s, 3H, Me), 1.81 (s, 3H, Me), 2.31 (t, 4H, J=6 Hz), 2.64 (m, 2H), 1.93 (dd, 1H, J=5, 15 Hz), 3.26 (s, 3H, OMe), 3.43 (s, 3H, OMe), 3.37 - 3.54 (m, 8H), 3.58 - 3.65 (m, 4H), 3.71 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.78 (s, 6H, OMe), 4.11 - 4.32 (m, 9H), 4.43 (m, 2H), 4.62 (m, 1H), 4.74 (m, 3H), 6.78 (m, 8H), 7.22 - 7.48 (m, 26H), 7.55 (d, 2H, J=7.9 Hz), 7.64 (d, 2H, J=8.3 Hz), 7.70 - 7.83 (m, 5H).

^{31}P NMR ($CDCl_3$) δ: 150.02, 150.84

<u>Example 18 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-isobutyryloxy piperidine-3-yl}adenine</u>

(Step 1) Preparation of (3R,4R,5R,6R)-N-benzhydryl-3-t-butyldimethylsilyloxy-4-isobutyryloxy-5,6-O-[(4-methoxy phenyl)ethylidene]piperidine

As a starting material, diastereomer B prepared from the step of Example 1 (or diastereomer A or mixture of A and B), was dissolved in 100 mL of anhydrous pyridine, to this resulting mixture were added triethylamine (2.2 mL, 20.74 mmol) and isobutyric anhydride (5.8 mL, 26.62 mmol), and this reaction mixture was stirred at 60°C for 1 day. The reaction mixture was cooled at room temperature, to the cooled reaction mixture was added 7 mL of methanol, and stirred for 30 min. The solvent was evaporated under reduced pressure, the residue diluted with ethylacetate, and washed with saturated sodium bicarbonate solution. The organic layer was separated, dried with sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography eluted with hexane/ethylacetate (10:1) to give 2.45 mg of the desired compound (55% yield).

^{1}H NMR ($CDCl_3$) δ -0.14 (s, 3H, Si-Me), -0.04 (s, 3H, Si-Me), 0.76 (s, 9H, Si-tBu), 1.27 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 1.35 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 1.54 (s, 3H, Me), 2.01 (dd, 1H, J=10.8, 10.8 Hz), 2.56 (ddd, 1H, J=4, 10.4, 10.4 Hz), 2.68 (m, 1H), 2.87 (dd, 1H, J=4.9, 11.4 Hz), 3.56 (d, 1H, J=8.4 Hz), 3.61 (d, 1H, J=6.9 Hz), 3.67 (dd, 1H, J=4.8, 10 Hz), 3.81 (s, 3H, OMe), 4.38 (dd, 1H, J=4, 10.4 Hz), 4.90 (dd, 1H, J=9.4, 9.4 Hz), 4.94 (s, 1H), 6.95 (d, 2H, J=8.7 Hz), 7.14 - 7.40 (m, 12H).

(Step 2) Preparation of (3R,4R,5R,6R)-N-benzhydryl-3-hydroxyl-4-isobutyryloxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.32 (d, 3H, J=6.4 Hz, $CH(CH_3)_2$), 1.34 (d, 3H, J=6.6 Hz, $CH(CH_3)_2$), 1.49 (s, 3H, Me), 1.96 (dd, 1H, J=10.9, 11 Hz), 2.55 (m, 1H), 2.77 (m, 1H), 3.08 (dd, 1H, J=5.1, 11.5 Hz), 3.52 - 3.80 (m, 3H), 3.87 (s, 3H, OMe), 4.47 (dd, 1H, J=4.1, 10.4 Hz), 4.67 (dd, 1H, J=9.2, 9.2 Hz), 4.97 (m, 1H), 6.97 (d, 2H, J=8.7 Hz), 7.14 - 7.44 (m, 12H).

(Step 3) Preparation of (3R,4R,5R,6R)-N-benzhydryl-3-methansulfonyl-4-isobutyryloxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.30 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 1.35 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 1.48 (s, 3H, Me), 2.17 (dd, 1H, J=10.8, 10.8 Hz), 2.57 (ddd, 1H, J=4, 10.2, 10.2 Hz), 2.74 (m, 1H), 2.90 (s, 3H, OSO_2CH_3), 3.22 (dd, 1H, J=5.2, 11.1 Hz), 3.62 (dd, 1H, J=10.4, 10.5 Hz), 3.68 (dd, 1H, J=9.2, 9.3 Hz), 3.87 (s, 3H, OMe), 4.45 (dd, 1H, J=4.2, 10.6 Hz), 4.68 (ddd, 1H, J=5.2, 5.3, 10.5 Hz), 4.98 (s, 1H), 5.06 (dd, 1H, J=9.4, 9.6 Hz), 6.96 (d, 2H, J=8.9 Hz), 7.15 - 7.42 (m, 12H).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-4-isobutyryloxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 0.97 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 1.50 (s, 3H, Me), 2.40 (m, 1H), 2.57 (dd, 1H, J=11.4, 11.6 Hz), 2.77 (m, 1H),

3.11 (dd, 1H, J=4.4, 11.3 Hz), 3.72 (dd, 1H, J=10.4, 10.4 Hz), 3.89 (s, 3H, OMe), 3.92 (m, 1H), 4.53 (dd, 1H, J=4, 10.5 Hz), 4.82 (ddd, 1H, J=4.3, 11.2, 11.3 Hz), 5.06 (s, 1H), 5.42 (dd, 1H, J=9.7, 10.4 Hz), 6.97 (d, 2H, J=8.7 Hz), 7.13 - 7.75 (m, 12H), 7.75 (s, 1H), 8.33 (s, 1H).

(Step 5) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-isobutyryloxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 0.97 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 1.51 (s, 3H, Me), 2.40 (m, 1H), 2.62 (dd, 1H, J=11.3, 11.4 Hz), 2.80 (m, 1H), 3.15 (dd, 1H, J=4.4, 11.3 Hz), 3.74 (dd, 1H, J=10.4, 10.5 Hz), 3.89 (s, 3H, OMe), 3.94 (dd, 1H, J=9.2, 9.2 Hz), 4.54 (dd, 1H, J=4, 10.5 Hz), 4.91 (m, 1H), 5.08 (s, 1H), 5.48 (dd, 1H, J=10, 10 Hz), 6.97 (d, 2H, J=8.8 Hz), 7.13 - 7.61 (m, 15H), 7.97 (s, 1H), 8.02 (d, 1H, J=7.3 Hz), 8.79 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-isobutyryloxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.75 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 0.90 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 2.33 (m, 1H), 2.71 - 2.76 (m, 2H), 3.26 (dd, 1H, J=4.1, 11.6 Hz), 4.14 - 4.23 (m, 2H), 4.34 (m, 1H), 4.91 (ddd, 1H, J=3.8, 11, 11 Hz), 5.37 (dd, 1H, J=9.2, 10.6 Hz), 5.62 (s, 1H), 7.17 - 7.40 (m, 10H), 7.52 (t, 2H, J=7.2 Hz), 7.60 (d, 1H, J=7.2 Hz), 7.90 (s, 1H), 8.02 (d, 1H, J=7.7 Hz), 8.79 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-isobutyryloxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.74 (d, 3H, J=7.1 Hz, $CH(CH_3)_2$), 0.91 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 2.34 (m, 1H), 2.63 (d, 1H, J=8.9 Hz), 2.75 (dd, 1H, J=11.3, 11.3 Hz), 3.25 (dd, 1H, J=4.2, 11.4 Hz), 3.79 (s, 6H, OMe), 4.23 (ddd, 1H, J=4.1, 10.8, 10.8 Hz), 5.20 (s, 1H), 5.33 (dd, 1H, J=9.5, 10.2 Hz), 6.81 (d, 2H, J=8.9 Hz), 6.83 (d, 2H, J=9 Hz), 7.22 - 7.41 (m, 19H), 7.52 (t, 2H, J=6.9 Hz),

7.59 (d, 1H, J=7.1 Hz), 7.98 (s, 1H), 8.02 (d, 1H, J=7.5 Hz), 8.81 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-isobutyryloxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.83 (d, 3H, J=7.3 Hz, $COCH(CH_3)_2$), 0.92 (d, 3H, J=6.9 Hz, $COCH(CH_3)_2$), 1.09 (d, 6H, J=6.7 Hz, $NCH(CH_3)_2$), 1.17 (d, 6H, J=6.9 Hz, $NCH(CH_3)_2$), 2.22 (m, 1H), 2.44 (t, 2H, J=6.5 Hz), 2.78 (dd, 1H, J=6.5, 12.5 Hz), 3.06 (dd, 1H, J=4.1, 12.6 Hz), 3.45 - 3.60 (m, 3H), 3.62 - 3.88 (m, 4H), 3.80 (s, 3H, OMe), 3.81 (s, 3H, OMe), 4.48 (m, 1H), 4.77 (s, 1H), 4.82 (m, 1H), 5.40 (t, 1H, J=5.4 Hz), 6.79 (d, 2H, J=8.8 Hz), 6.80 (d, 2H, J=8.8 Hz), 7.11 - 7.56 (m, 22H),8.05 (d, 2H, J=7.2 Hz), 8.71 (s, 1H), 8.76 (s, 1H).

^{31}P NMR ($CDCl_3$) δ 150.56, 150.83.

Example 19 : The synthesis of oligomers

Using the obtained monomer in Examples 1 to 18, an antisense oligomer was prepared as follows. All

oligomers was synthesized trityl-on with an Applied Biosystem 392 (DNA/RNA synthesizer) (1 μmol scale). Time of general condensation is 1 min, whereas was 10 min in case of nucleotides containing azasugar having other group except for hydrogen at position 4. Solid support and protective group were removed by heating with ammonium hydroxide for 17 hours at 55°C , and this solution was freeze-dried by adding 5 drops of triethylamine every hour in order to inhibit deprotecting of the protective group. The residue was dissolved in 1 mL of 100 mM triethylammonium acetate (TEAA) at pH 7, and purified by reversed phase high performance liquid chromatography (RP-HPLC, Hamilton PRP-1, 300 mm X 7 mm, 18 - 28% acetonitrile/100 mM TEAA, pH 7, monitored at 260 nm). The desired fraction was freeze-dried, the residual TEAA was removed through adding twice 1 mL of distilled water, and freeze-dried. The residual solid was thoroughly dissolved by vortexing in 0.3 mL of 80% acetic acid, and dimethoxytrityl group was removed by incubating at room temperature for 20 min. 0.3 mL of Ethanol was added to the above solution to remove acetic acid, and freeze-dried. 1 mL of Distilled water was added to the residue, and dissolved by vortexing. 1 mL of Ether was added to the resulting solution, and stirred well by vortexing. Ether layer was removed by using pipet, 1 mL of ether was again added, and stirred well. This

procedure was repeated twice. After freeze-drying of water layer, 1 mL of distilled water was added to the residue, and the resulting solution was quantified by UV absorbance at 260 nm at 70°C . The extinction coefficients (at 260 nm) of natural nucleotides used for calculations were as follows: dAMP : 15200; dCMP : 7700; TMP : 8830; dGMP : 11500. The extinction coefficients of nucleotides having azasugar were considered as the same value as that of natural nucleotides. All oligomers were characterized by enzyme digestion followed by HPLC (Hewlett Packard, ODS hypersil, C-18; 20 mM K_2HPO_4, pH 5.6 (A), MeOH (B), 100% A to 40% B, 20 min) and laser desorption mass spectrometry.

Example 20 : Hybridization properties of oligomer : melting studies

UV absorbance versus temperature profiles was measured on a Beckmann DU 650 spectrophotometer with Beckmann high performance temperature controller. Nitrogen gas was passed over the cell at less than room temperature to avoid the condensation of moisture. The temperature of the cell holder was increased from 5°C to 90°C in 1°C increments at a heating rate of 1°C /min. 2.5 μM of antisense oligomer and RNA, and buffer (100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7)

were employed. Melting temperature (Tm) was determined by first derivative of absorbance versus temperature curve. Reverse melting temperature was also measured (90°C to 5°C , heating rate of 1°C /min), and found to give reverse Tm within ± 1°C of forward Tm. Tm of RNA complementary with azasugar-containing antisense oligomer was compared to that of a control, represented by SEQ ID NO 1. As shown in table 1 and table 2, the underlined nucleotide indicates a nucleotide monomer subsituted with R^1 and R^2 of the present invention. For example, the 5th nucleotide in the oligomer represented by SEQ ID NO 3., substitutes R^1 and R^2 with β-OME and benzhydryl, respectively. According to tables 1 and 2, a high value of Tm indicates strong binding affinity

<Table 1> Tm of antisense oligomers

substituents		oligomer	SEQ	Tm	ΔTm
R^1	R^2		ID	(RNA)	
-	-	5'-dAGG GAG AGA AAG-3' 5'-rCTT TCT CTC CCT-3'	NO 1 NO 2	34°C	-
β-OMe	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	NO 3	36°C	+2°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 4	39°C	+5°C
		5'-**A**GG G**A**G AGA AAG-3'	NO 5	40°C	+6°C
β-OMt	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	NO 6	36°C	+2°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 7	38°C	+4°C
β-OMt OMe	Benzhydryl	5'-AGG G**A**G AGA AAG-3	NO 8	37°C	+1°C
H	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	NO 9	34°C	0°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 10	34°C	0°C
α-OMe	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	NO 11	36°C	+2°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 12	37°C	3°C

β-OMe	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	NO 13	33°C	-1°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 14	34°C	0°C
β-OMe	Me	5'-AGG G**A**G AGA AAG-3'	NO 15	28°C	-6°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 16	20°C	-14°C
β-OMe	n-propyl	5'-AGG G**A**G AGA AAG-3'	NO 17	31°C	-3°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 18	26°C	-8°C
β-OMe	Benzyl	5'-AGG G**A**G AGA AAG-3'	NO 19	33°C	-1°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 20	33°C	-1°C
β-OMe	4-cyanobenzyl	5'-AGG G**A**G AGA AAG-3'	NO 21	32°C	-2°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 22	31°C	-3°C
β-OMe	4-fluoro-benzyl	5'-AGG G**A**G AGA AAG-3'	NO 23	33°C	-1°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 24	33°C	-1°C
β-OMe	4-methoxy-benzyl	5'-AGG G**A**G AGA AAG-3'	NO 25	31°C	-3°C
		5'-AGG G**A**G AG**A** AAG-3'	NO 26	31°C	-3°C
β-OMe	Benzhydryl	5'-AGG GAG A**G**A AAG-3'	NO 27	25°C	-9°C
		5'-AG**G** GAG A**G**A AAG-3'	NO 28	20°C	-14°C
β-OH	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	NO 29	31°C	-3°C

<Table 2> Tm of antisense oligomer

substituents		oligomer	SEQ ID	Tm (RNA)	ΔTm
R^1	R^2				
-	-	5'-rAGG GAG AGA AAG-3' 5'-dCTT TCT CTC CCT-3'	NO 1 NO 2	54°C	-
β-OMe	Benz-hydryl	5'-CTT T**C**T CTC CCT-3'	NO 30	34°C	-20°C
β-OMe	Benz-hydryl	5'-CTT TCT C**T**C CCT-3'	NO 31	46°C	-8°C
β-OMe	Me	5'-CTT TCT C**T**C CCT-3'	NO 32	42°C	-12°C
β-OMe-	H(Fmoc protec-ting group)	5'-CTT TCT C**T**C CCT-3	NO 33	40°C	-14°C

As indicated in Table 1 and 2, all oligomers showed strong binding affinities for RNA than natural

type DNA helix, especially 1-6°C increment in Tm of the oligomers having monomers of Example 1, 2, 3 or 5, indicated that the oligomers bind with greatest affinity to a complementary strand of RNA.

Thus, the oligomers of the present invention can be used as antisense oligomers with great binding affinity for RNA.

Example 21 : Stability of oligomers against nuclease.

Into an eppendorf tube were added 0.15 OD (optical density) of each oligomer, 0.03 units of snake vernom diesterase and 0.18 units of alkaline phosphatase (Buffer : 100 mM $MgCl_2$, 4 μL: 0.25 M Tris HCl, pH 8.1, 8 μL: distilled water 13 μL). The reaction mixture was incubated at 37°C , and aliquots were removed every 30 min. The amount of oligomer degradation associated with each aliquot was determined by HPLC (Hamilton PRP-1, 300 mm x 7 mm, 260 nm). Under these conditions, all of the natural oligomers were degraded within 30 min, whereas the three oligomers of Example 1 were not completely degraded even after 4 hours. The monomers of the present invention did not show its peak on HPLC except for segments of oligomers partially degraded. This result demonstrated that the above nucleases did not degrade DNA segments linked to the monomers of the present invention.

Example 22 : Acute toxicity test with rat via parental route

Specific pathogen-free (SPF) SD-rats which were six weeks old, were tested for acute toxicity. Suspensions of the compounds of the Example 1 - 19 in 0.5% methyl cellulose were orally administered once at a dose of 1 g/kg/15ml to the rats, which were grouped in twos. After the administration, the animals were observed as to their death, clinical symptoms and weight change, and serological and serobiochemically tested. An autopsy was made over the rats with the naked eye to observe whether their abdominal and thoracic organs were damaged. Neither sudden death nor noticeable clinical symptoms were detected from all of the animals administered with the compounds of interest. In addition, no toxic signs were observed in weight change, serologic test, serobiochemical test, and corpse examination. The compounds tested caused no toxic changes rats over the rats to the dose of 500 mg/kg and thus, found to be safe compounds with a lethal dose (LD_{50}) of at least 500 mg/kg when being administered via an oral route.

INDUSTRIAL APPLICABILITY

The present invention relates to antisense monomers and oligomers which can inhibit transcription for the production of disease-inducing proteins. The antisense monomers and oligomers of the present invention have higher binding affinity for RNA, the target of general antisense drugs, than that for DNA. In addition, they have increased nuclease resistance and improved permeability of cell membrane.

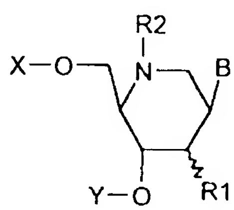
The monomers and oligomers of the present invention can be used for antisense therapy inhibiting the expression of genes inducing diseases, hybridization of gene cloning and reagents for diagnosis. They also provide useful tools for the investigation of proteins.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

What is Claimed is

1. Modified nucleotide monomers represented by the formula 1, in which five-membered ribose, sugar of natural nucleotide, is substituted with azasugar of six-membered ring.

Formula 1



(α or β)

Wherein

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; araalkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaraalkyl such as fluorobenzyl; cyanobenzyl;

alcoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

(4) X is hydrogen or hydroxy protecting group, and

(5) Y is hydrogen, phosphate, activated phosphate, activated phosphite or solid support.

2. The nucleotide monomers according to claim 1, wherein R1 is selected from the group consisting of hydrogen, methoxy, ethoxy and methoxyethoxy.

3. The nucleotide monomers according to claim 1, wherein R2 is selected from the group consisting of diphenylmethyl, methyl, *t*-butyl, benzyl, cyanobenzyl, fluorobenzyl, methoxybenzyl and fluorenyl (Fmoc).

4. The nucleotide monomers according to claim 1, which are represented by the formula 1 and is selected from the group comprising:

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 1);

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-

dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl} adenine (the compound of Example 2);

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxyethoxypiperidine-3-yl}adenine (the compound of Example 3);

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethylpiperidine-3-yl}adenine (the compound of Example 4);

6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 5);

6-N-benzoyl-{(3S,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl} adenine (the compound of Example 6);

6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-[(2-cyano ethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyl trityloxymethyl-4-methoxypiperidine-3-yl}adenine (the compound of Example 7);

6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-[(2-cyano ethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyl trityloxymethyl-4-methoxypiperidine-3-yl}adenine (the compound of Example 8);

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-[(2-cyano

ethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine (the compound of Example 9);

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 10);

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 11);

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 12);

4-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} cytosine (the compound of Example 13);

2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} guanine (the compound of Example 14);

{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrithyloxymethyl-4-methoxypiperidine-3-yl}thymine(the compound of Example 15);

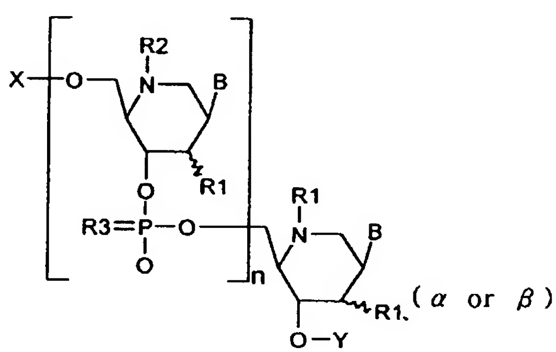
{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine (the compound of Example 16);

{(3R,4R,5R,6R)-N-fluorenyl-5-[(2-cyanoethoxy)N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine (the compound of Example 17); and

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-isobutyryloxypiperidine-3-yl}adenine (the compound of Example 18);

5. Antisense oligomers represented by the formula 2, which are prepared with the nucleotide monomers of claim 1 as a part or whole of oligonucleotide.

Formula 2



Wherein,

n is 0 to 30,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; araalkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaraalkyl such as fluorobenzyl; cyanobenzyl; alcoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

(4) R^3 is oxygen or sulfur,

(5) X is hydrogen or hydroxy protecting group, conjugate group or oligonucleotide, and

(6) Y is hydrogen, phophate, active phosphate, active phosphite, solid support, conjugate group or oligonucleotide.

6. The antisense oligomers according to claim 5,

wherein R1 is selected from the group consisting of hydrogen, methoxy, ethoxy and methoxyethoxy.

7. The antisense oligomers according to claim 5, wherein R2 is selected from the group consisting of diphenylmethyl, methyl, *t*-butyl, benzyl, cyanobenzyl, fluorobenzyl, methoxybenzyl and fluorenyl (Fmoc).

8. Chimeric oligomers, which have the monomers of claim 1 or the antisense oligomers of claim 5 at both ends of the molecules, and phosphodiesters or phosphothioate oligonucleotides in their middle.

9. A method for preparing nucleotide monomers of claim 1, which comprises the following steps:

(1) Obtaining a ketone compound through acidification of glucose with a protecting group;

(2) Removing the protecting group in the compound of step (1) using an acidic resin;

(3) Obtaining the azasugar of six-membered ring by ring formation of the compound of step (2);

(4) Protecting a primary alcohol group at position C-6 and secondary alcohol at position C-5 of the compound of step (3);

(5) Protecting a secondary alcohol at position C-3 of the compound of step (4), followed by alkylation of alcohol at position C-4;

(6) Removing and mesylating the protecting group at C-3 position of the compound of step (5);

(7) Preparing nucleosides by condensation of the compound of step (6) with base; and

(8) Linking phosphate groups to the nucleosides of (7).

10. A process for preparing antisense oligomers of claim 5, which comprises:

(1) Substituting dimethoxytrithyl group for a primary hydroxyl group linked to the sugar of a nucleotide monomer, phosphoramidite group for a secondary alcohol group, and to protect nucleobases except thymine, with an appropriate protecting group;

(2) Performing condensation reaction of the monomer of step (1) linked to solid support with a oligonucleotide;

(3) Removing the solid support and protecting group from the oligomer of step (2);

(4) Removing a 5'-hydroxy protecting group from the oligomer.

11. The process for preparing antisense oligomers according to claim 10, wherein condensation of step (2) is characterized by having the nucleotide monomer linked to the solid support at position 3'.

12. The process for preparing antisense oligomers according to claim 10, wherein the condensation of step (2) is characterized by having a nucleotide monomer linked to the solid support at positions except 3'-terminus, prepared via standard phoshoamidite process using a DNA synthesizer.

13. Pharmaceutical compositions containing the antisense oligomers of claim 5 or the chimeric oligomers of claim 8 as active ingredients, which are effective for inhibition or prevention of proteins syntheses.

14. Pharmaceutical compositions containing the antisense oligomers of claim 5 or the chimeric oligomers of claim 8 as active ingredients, which are effective for the treatment of hepatitis of viral or bacterial origin, cancers and immune diseases.

ABSTRACT OF THE DISCLOSURE

The present invention relates to antisense monomers and oligomers which can inhibit transcription for the production of disease-inducing proteins. The antisense monomers and oligomers of the present invention have greater binding affinity for RNA, a target of general antisense medicine, than that for DNA. In addition, they have nuclease resistance, and can improve permeability of cell membrane.

The monomers and oligomers of the present invention can be used for antisense therapy inhibiting the expression of disease-inducing genes, hybridization of gene cloning and reagents for diagnosis. They also provide useful tools for the proteins.

DECLARATION FOR PATENT APPLICATION- SOLE OR JOINT

Docket No . 428.1011

As a below named inventor, I hereby declare that·

My residence, post office address and citizenship are stated below next to my name

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention titled·

NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZARSUGAR AND ANTISENSE OLIGOMERS THEREOF

the specification of which is (check one)

☒ attached hereto, or

☐ was filed on ____________ as Application Serial No ____________ and was amended on ____________ (if applicable).

I HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS.

I ACKNOWLEDGE THE DUTY TO DISCLOSE INFORMATION WHICH IS MATERIAL TO THE EXAMINATION OF THIS APPLICATION IN ACCORDANCE WITH TITLE 37, CODE OF FEDERAL REGULATIONS, SECTION 1.56(a)

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for the patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
1999-26947	Republic of Korea	05/07/1999	☒ Yes ☐ No
PCT/KR00/00713	Republic of Korea	03/07/2000	☒ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. Section 119(3) of any United States provisional applications listed below

(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
			☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States applications listed below and, INSOFAR AS THE SUBJECT MATTER OF EACH OF THE CLAIMS OF THIS APPLICATION IS NOT DISCLOSED IN THE PRIOR UNITED STATES APPLICATION IN THE MANNER PROVIDED BY THE FIRST PARAGRAPH OF TITTLE 35, UNITED STATES CODE, SECTION 112, I ACKNOWLEDGE THE DUTY TO DISCLOSE MATERIAL INFORMATION AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, SECTION 1 56(a) WHICH OCCURRED BETWEEN THE FILING DATE OF THE PRIOR APPLICATION AND THE NATIONAL OR PCT INTERNATIONAL FILING DATE OF THIS APPLICATION

(Application Serial Number)	(Filing Date)	(STATUS Patented, Pending, Abandoned)

POWER OF ATTORNEY

As named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Offic connected herewith (List name and registration number)

(Name)	(Registration Number)
Michael N. Mercanti	33,966
Richard S Roberts	27,941
Laurence Manber	35,597
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DECLARATION FOR PATENT APPLICATION- SOLE OR JOINT (Continued) Docket No.: 428.1011

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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